



**PRE- AND POST-HARVEST APPLICATION OF SALICYLIC
ACID AND SODIUM NITROPRUSSIDE FOR MAINTAINING
POSTHARVEST QUALITY OF MANGO**

SI THU WIN

**MASTER OF SCIENCE
IN
POSTHARVEST TECHNOLOGY AND INNOVATION**

**SCHOOL OF AGRO-INDUSTRY
MAE FAH LUANG UNIVERSITY**

2022

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Si Thu Win

Thesis Title	Pre- and Post-harvest Application of Salicylic Acid and Sodium Nitroprusside for Maintaining Postharvest Quality of Mango
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Degree	Master of Science (Postharvest Technology and Innovation Program)
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ABSTRACT

Mango fruit have a high nutritional value and are beneficial to health. However, losses frequently occur after harvest, because they are perishable. Salicylic acid (SA) has been used to preserve fruit quality and maintain their nutritional contents. Therefore, this study was conducted to investigate the effects of 2 mM SA on the physicochemical properties, bioactive compounds, antioxidant and anti-inflammatory activities of mango fruit (*Mangifera indica* L. cv 'Nam Dok Mai Si Thong'. For this purpose, mango fruit were given preharvest (Pre SA) or postharvest applications of SA (Post SA) and their combination (Pre + Post SA); the fruit were stored at 13 °C for 20 days. Weight loss, decay, and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity were maintained in SA-treated fruit. The Pre + Post SA treatment was superior in delaying fruit ripening, maintaining lower soluble solids contents and higher total acidity. In addition, total phenolic compounds (TPC), ferric reducing antioxidant power (FRAP), and free radical scavenging activity of anti-inflammatory substances such as nitric oxide as well as hyaluronidase inhibition was higher in the Pre + Post SA treatment throughout storage. Therefore, both pre- and post-harvest SA

treatments are recommended for preserving the quality of mango fruit and for maintaining their nutritional properties for human health.

Nitric oxide (NO) has been reported to delay ripening and maintain fruit quality as well as SA. In this study, the effects of applying 1 mM sodium nitroprusside (SNP), a NO donor, on the ripening-related properties, bioactive compounds, and antioxidant and anti-inflammatory properties of 'Nam Dok Mai Si Thong' mango were investigated. For this study, mango fruits were treated at preharvest with SNP (Pre SNP), postharvest with SNP (Post SNP), and at preharvest and postharvest with SNP combinations (Pre + Post SNP). All mango fruits were stored at 13°C, with 80–85% RH for 20 days before quality determination. SNP-treated fruit maintained the changes in total color differences, firmness, soluble solids content (SSC), and total acidity (TA), and averaged 4% less weight loss and 60% lower decay than those of the control fruits through day 20. In addition, Pre + Post SNP showed significantly increased TPC, DPPH radical scavenging activity, FRAP, and anti-inflammatory activity such as hyaluronidase inhibition activity compared to the other treatments. The application of SNP in pre and postharvest treatments enhanced mango fruits' bioactive compounds, antioxidants, and anti-inflammatory activities.

Keywords: Antioxidant, Anti-inflammatory, Mango Fruit, Nitric Oxide, Salicylic Acid, Sodium Nitroprusside

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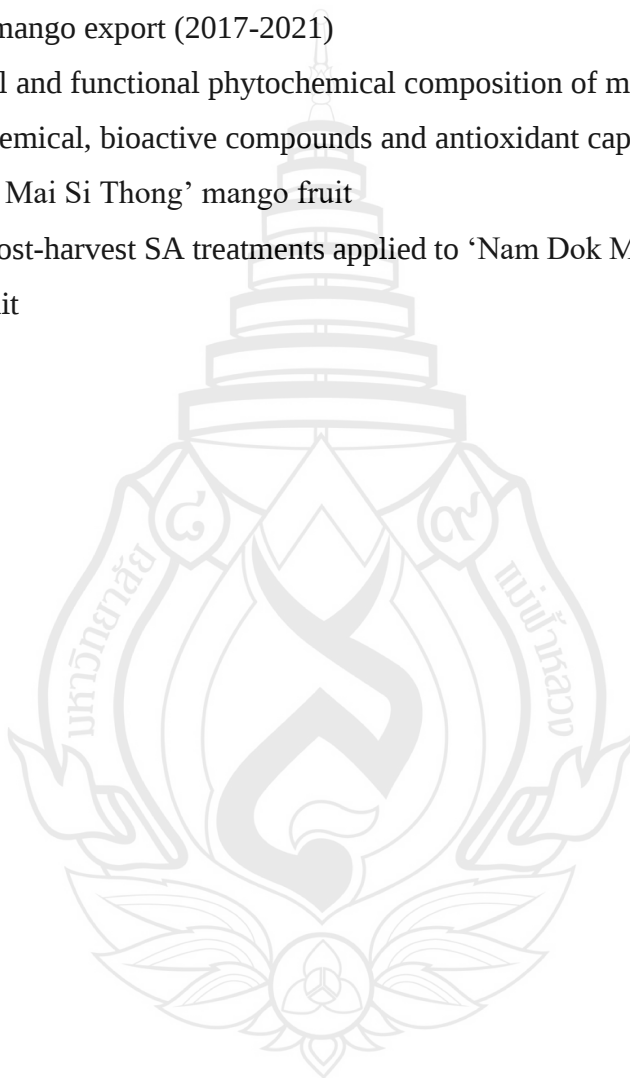
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ABBREVIATION AND SYMBOL

SA	Salicylic Acid
NO	Nitric Oxide
SNP	Sodium Nitroprusside
TA	Total Acidity
SSC	Soluble Solid Content
N	Newton
TE	Trolox Equivalent
TPC	Total Phenolic Content
GAE	Gallic Acid Equivalent
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
FRAP	Ferric Reducing Antioxidant Power
DW	Dry Weight
UV-C	Ultra Violet C
HA	Hyaluronidase
USDA	United States Department of Agriculture
SE	Standard Error
CRD	Completely Randomized Design

CHAPTER 1

INTRODUCTION

1.1 Introduction to the Research Problem and its Significance

Mango is the second most important tropical fruit in horticulture and is valued for its delicate taste, pleasant aroma, and economic benefits. Furthermore, mango has a high nutritional value due to its phytochemical composition (Maldonado-Celis et al., 2019). It is grown in 85 countries worldwide, including Asian countries. Among them, Thailand was the 3rd highest producer in the world in 2019–2020, and ‘Nam Dok Mai Si Thong’ mango is one of the leading export products of Thailand (Lebaka et al., 2021; Noiwan et al., 2017). The skin of mango is initially green and becomes golden-yellow as it ripens. This variety is fragrant, sweet, and juicy, and has little fibrous tissue (Chimvaree et al., 2020). Although mango has many benefits, its quality and nutritional value are difficult to maintain, because fruit can ripen rapidly in 7 to 9 days at ambient temperature. During ripening, fruit undergo various biochemical changes including, climacteric respiration, ethylene production, changes in carbohydrates, organic acid, phenolic compounds and production of aroma volatile compounds (Rees et al., 2012; Singh, et al., 2013b).

Postharvest treatments such as salicylic acid, nitric oxide, hot water, UV-C irradiation, controlled atmosphere and coatings are applied to delay ripening and maintain the quality and nutritional value in mango fruit (Bambalele et al., 2021). Moreover, pre-harvest factors also influence postharvest quality of mango fruit. During fruit development on the mango tree, disease infections and extreme environmental conditions are the main pre-harvest factors that affect postharvest quality. Salicylic acid and nitric oxide are considered as plant signal molecule and involved in plant growth and defense responses (Mansouri, 2012).

SA, also known as ortho-hydroxybenzoic acid, is an endogenous signaling molecule that modulates stress responses and plant physiological processes, such as photosynthesis, heat production, stomatal conductance, transpiration, disease resistance, seed germination, crop yield, and glycolysis (Klessig & Malamy, 1994). In recent years, exogenous applications of SA have been used as pre- and post-harvest treatments. TPC, and the antioxidant capacity of peach fruits can be improved by postharvest application of 2 mM SA, with no negative effects on flavor or appearance (Khademi & Ershadi, 2013). This concentration also had the greatest effect on reducing weight loss, fruit softening, disease incidence of ‘Chausa’ mango, and increased concentrations of bioactive compounds and antioxidant capacity (Barman & Asrey, 2014). Pre-harvest SA applications have also maintained the TPC and total antioxidant activity of tomatoes (Baek et al., 2021). In addition, pre-harvest applications of SA also helped reduce weight loss and firmness of grapes (Loay, 2017) and lemons (Serna-Escolano et al., 2021), and maintained postharvest quality by enhancing the preservation of SSC, TA, and ascorbic acid and total antioxidant contents of ‘Amrapali’ mango fruit (Reddy & Sharma, 2016). In addition, pre- and post-harvest applications of SA reduced internal browning and maintained the quality of winter pineapple fruit (Lu et al., 2011), and have also maintained the quality of tomatoes (Baninaiem et al., 2016).

NO is a hydrophobic, highly diffusible gaseous molecule that regulates a wide range of plant growth and developmental processes, including germination, metabolism, signal transmission, flowering, and senescence (Besson-Bard et al., 2008). It reduces ethylene production and, consequently, can have anti-ripening, fruit softening, and senescence properties (Leshem & Pinchasov, 2000; Manjunatha et al., 2012). As a result, it can be used to increase shelf life (Pareek, 2017), and exogenous applications of sodium nitroprusside (SNP), an NO donor, have been applied as pre- and post-harvest treatments and have been shown to significantly delay the ripening in apple fruit during storage (Cheng et al., 2009) through their effects on ethylene production (Deng et al., 2013) and in pear fruit, SNP treatment reduced losses in fruit mass, maintained color and high firmness, and preserved total soluble solids, acidity, phenolic content, and ascorbic acid, thereby maintaining the fruit quality (Adhikary et al., 2021). In addition, SNP treatment of litchi fruit delayed the weight loss and fruit

decay and improved the levels of total phenolics and antioxidant capacity, which were 50% and 31% higher, respectively, in relation to untreated fruits (Barman et al., 2014). Postharvest application of 1 mM SNP applied to ‘Nam Dok Mai No.4’ significantly suppressed ethylene production and respiration compared to the control fruit during storage (Tran et al., 2015). However, the effect of both pre- and post-harvest applications of SA and SNP on mango fruit has been little studied.

The health benefits of fruits are partly due to compounds that have antioxidant capacity and the ability to defeat oxidative stress by neutralizing the excess of oxidative species. A variety of compounds contribute to the maintenance of human health. Antioxidants in fruits help the human body’s defense systems by lowering the steady production of prooxidants, such as reactive oxygen species and their products. Cardiovascular disease, cerebrovascular illness, and cancer can all be caused by oxidative stress (Baninaiem et al., 2016). NO damages membranes by causing lipid peroxidation and lipoprotein oxidation, both of which can contribute to inflammatory illnesses, atherosclerosis, cancer, cardiovascular disease, and aging (Boora et al., 2014); NO scavenging assay are often used to measure anti-inflammatory activity of fruit. In addition, NO scavengers and hyaluronidase inhibitors are effective in reducing allergic reactions and inflammation (Furusawa et al., 2011), and work by inhibiting hyaluronidase, an enzyme that breaks down hyaluronic acid and chondroitin sulfate. To date, the effect of SA and SNP applications on anti-inflammatory activities, such as nitric oxide scavenging activity and hyaluronidase inhibitory activity (HA), have never been reported in mango fruits.

Therefore, the present study aimed to evaluate the ripening related properties, bioactive compounds, and antioxidant and anti-inflammatory properties of ‘Nam Dok Mai Si Thong’ mango by using 2 mM SA and 1 mM SNP in pre- and post-harvest applications, and their combination.

1.2 Objectives

To investigate the effectiveness of pre- or postharvest application including combination of pre- and postharvest for SA (experiment 1) and NO (experiment 2) on

postharvest quality of ‘Nam Dok Mai Si Thong’ mango by measuring physical, phytochemicals, bioactive compounds, antioxidant and anti-inflammatory properties.

1.3 Scope of Research

Experiments	Application	Treatments	Measurements
• Experiment 1 [SA application] • Experiment 2 [SNP application]	• Pre – harvest treatment (spraying method) • Postharvest treatment (dipping method)	• Control • Pre – harvest treatment • Postharvest treatment • Pre - and Post harvest treatment	• Physical and physicochemical quality • Fruit decay • Bioactive compounds • Antioxidant activity • Anti-inflammatory activity

Figure 1.1 Scope of research

1.4 Research Output

To obtain an effective application (i.e., pre-, post-harvest or combination between pre- and post-harvest) of SA or NO for maintaining quality of ‘Nam Dok Mai Si Thong’ mango.

1.5 Research Outcomes

1.5.1 Apply chemical treatment before and after harvest using a simple application in postharvest technology.

1.5.2 Promote the safety level of chemical treatment application to farmers

1.5.3 Enhance quality of ‘Nam Dok Mai Si Thong’ by improving the bioactive compounds, antioxidants and anti-inflammatory properties and extending their storage life.

1.6 Location of Research

Singha Park, Mango Orchid, Mae Kon Sub District, Chiang Rai Province, Thailand

Postharvest Technology Laboratory, Scientific and Technological Instruments Center, Mae Fah Laung University, Chiang Rai, Thailand.



CHAPTER 2

LITERATURE REVIEW

2.1 Mango

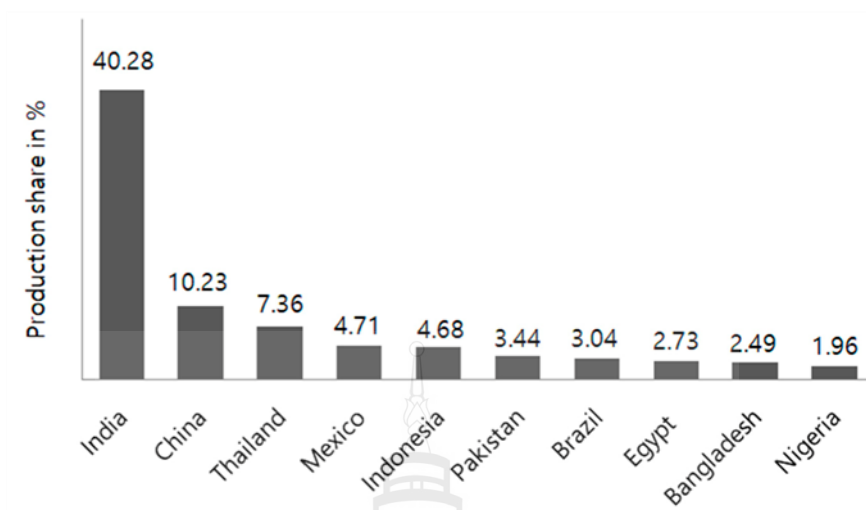
Mango (*Mangifera indica* L.) is a tropical fruit that originated in Southeast Asia (Kostermans & Bompard, 1993) and it belongs to the Anacardiaceae family. It is now cultivated all region of the world. Mangos have a high nutritional content and are commercially available in a variety of countries. Mangoes are consumed in a various form, including fresh fruit and in processed form as juice, powder, pickles, and jam. In Asia, mango is also a popular dessert fruit. Southeast Asian countries having large plantations for both domestic and export markets include the Philippines, Thailand, Myanmar, and Indonesia. The 'Nam Dok Mai Si Thong' variety, is one of most popular export mango fruits of Thailand, as well as a popular fruit in the local market. It has higher flavonoids, phenolic and antioxidant capacity when compared with other Thai mango cultivars such as Nuan Chan, Thongdum and Yaigrum (Rumainum et al., 2018) and also have unique flavor and fragrance. The skin of the 'Nam Dok Mai Si Thong' is typically green, but turns golden-yellow as it ripens (Chimvaree et al., 2020).



Figure 2.1 ‘Nam Dok Mai Si Thong’ mango fruit

2.2 Economic Importance

Mangoes are grown in different ways, depending on size, shape, sweetness, skin and flesh color. It has great flavor and aroma with a wonderful balanced of sweetness and acidity and high nutritional value. Mango production continues to rank it as the most popular tropical fruit in the 21st century (National Mango Database, 2020). Mango is grown in 85 countries around the world, with Asia accounting for 80 percent of total production, including India, China, Thailand, and Indonesia. Thailand is the third-largest producer of mango fruits accounting for 7.36 % while India and China are the first and second-largest producers, accounting for 40.28% and 10.23% respectively (Figure 2.2).



Source National Mango Database (2020)

Figure 2.2 Production share (%) of the top ten mango-producing countries in 2019–2020

Thailand mango export income has increased almost twice in 2021 (5997.06 million baht) when compared with 2017 (3326.8 million baht), and the amount of export has increased even more than twice the amount (59138.59 tons to 149151.48 tons) as seen in Table 2.1. Hong Kong, the United States, and Japan are Thailand's top mango export markets.

Table 2.1 Thailand mango export (2017-2021)

Years	Volume (Tons)	Value (million baht)
2017	59138.56	3326.8
2018	94102.39	4383.71
2019	85182.87	4067.11
2020	117656.52	4602.18
2021	149151.48	5997.06

Source Thairath Online (2022)

2.3 Nutritional Value

Mango fruit is an important source of macronutrients such as carbohydrates, lipids, fatty acids, protein, amino acids, and organic acids. Mango is also high in micronutrients including vitamins and minerals. Phenolic compounds, flavonoids and other polyphenols, chlorophyll, carotenoids, and volatile compounds are examples of non-nutrient substances (Maldonado-Celis et al., 2019). Sugars (glucose, fructose, and sucrose) as well as other carbohydrates like starch and pectin are abundant in ripened mango fruit (Table 2.1) (Bello-Pérez et al., 2007). From a nutritional and flavor standpoint, these are all important components. About 15% of total sugars are found in the flesh of a ripe mango. Starch is the most important from a quantitative point of view in the unripe mango fruit. Starch is hydrolyzed to glucose during ripening, and glucose phosphate enters the hexose phosphate pool after phosphorylation, fueling a "futile cycle" of sucrose synthesis and degradation that regulates glucose, fructose, and sucrose levels. As a result, glucose, fructose, and sucrose levels rise during ripening (Arbuthnot, 2002; Geigenberger & Stitt, 1991; Kuete, 2017). Mango fruit acidity is mostly due to the presence of citric and malic acids (Table 2.1) (Matheyambath et al., 2016).

Vitamin C and vitamin A are dominant (Table 2.2), suggesting that regular consumption of mango fruit can provide the necessary dietary requirements of these vitamins (Diet, 2003). Vitamin C level changes as mangos ripen; it is higher in less ripe mangos than in fully ripe mangos (Matheyambath et al., 2016). The vitamin C decrease may be due to the involvement of different metabolic pathways such as ethylene, the oxalate, and tartrate biosynthesis because vitamin C is a coenzyme of their respective enzymes (Singh et al., 2011). Calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, manganese, and selenium are among the minerals found in the edible portion of mango fruits (Table 2.2).

Table 2.2 Nutritional and functional phytochemical composition of mango pulp

Compound (per 100g)	Values
Water (g)	83.46
Energy (kcal)	60
Carbohydrate (g)	14.98
Protein (g)	0.82
Total lipid (fat) (g)	0.38
Total sugar (g)	13.66
Minerals(mg)	Values
Calcium (Ca)	11
Iron (Fe)	0.16
Magnesium (Mg)	10
Phosphorus (P)	14
Potassium (K)	168
Sodium (Na)	1
Zinc (Zn)	0.09
Copper (Cu)	0.04–0.32
Selenium (Se)	0–0.6
Organic acids	Values
Citric acid (%)	0.7
Malic acid (%)	0.5

Source Lebaka et al. (2021) and U.S. Department of Agriculture (2019)

Phenolic and Carotenoid

Bioactive compounds such as vitamins, phenolics, and carotenes act as precursors of molecules and secondary metabolites involved in the plant defense systems, such as antifungals, antibiotics, and antivirals (Baenas et al., 2014). Mango pulp contains hydroxybenzoic and hydroxycinnamic acid derivatives, which are the two most common types of phenolic acids in plants. These phenolic acids can be found in their natural state or in conjugated forms with glucose (Burton-Freeman et al., 2017; Mattila & Kumpulainen, 2002). The hydroxybenzoic acids that have been detected in the mango pulp are gallic acid, vanillic, syringic, protocatechuic acid, p-hydroxybenzoic acids, while the hydroxycinnamic acid derivatives are p-coumaric, chlorogenic, ferulic, and caffeic acids (Ediriweera et al., 2017; Masibo & He, 2008). Moreover, because of their ability to neutralize free radicals naturally formed during

ripening processes or senescence of fruits and to reduce pathogen attack, the concentration of phenolic compounds (polyphenols and phenolic acids) fluctuates during development until maturity (Matheyambath et al., 2016; Palafox-Carlos et al., 2012).

During mango ripening, the chlorophyll (II) present in the unripe stage is degraded, as is the uncovering of previously existing colors and the biosynthesis of anthocyanins and carotenoids. The degree of sun exposure determines the formation of anthocyanins, which contribute to the red coloring of the mango peel and carotenoids can be found in fruit at a later stage of ripening (Medlicott et al., 1986). Color pigments, termed carotenoids, are responsible for the characteristic color of mango skin and pulp. Provitamin A activity, antioxidant activity, cell communication, immune function enhancement, UV skin protection, accessory pigments for light harvesting, and defense against photo-oxidative damage are just a few of the roles that carotenoids play in the biological processes of both plants and animals (Van den Berg et al., 2000). Evidence has been presented that individual with low carotenoid intake and/or low carotenoid blood levels have an increased risk of degenerative diseases. In a number of these diseases, free radical damage is thought to play a role in pathophysiology (Van den Berg et al., 2000). Carotenoids can help prevent free radical damage by acting as antioxidants because of their ability to quench radical species. Thus, carotenoids have been noted as being the most abundant micronutrients found in cancer-preventative foods (Cano & de Ancos, 1994).

2.4 Antioxidant Activity

Through oxygen consumption, numerous enzymatic systems in the human body produce reactive oxygen species (ROS), such as superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide (Atmani et al., 2009). Under oxidative stress, over a hundred human disease problems, including cancer, cardiovascular disease, aging, and neurological diseases, have been associated with large quantities of these ROS (Bagchi et al., 2000). Therefore, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in

order to balance the ROS. Fruit is one of the main dietary sources of a variety of antioxidant phytochemicals for humans. Mangoes, as well as citrus fruits, can be considered a good source of dietary antioxidants such as ascorbic acid, carotenoids, and phenolic compounds. Polyphenols are natural substances in plants that are antioxidants with the potential to protect the body from disease (Ma et al., 2011). Ferric reducing antioxidant power (FRAP), 2,2-azino-di-(3-ethylbenzothiazoline-sulphonic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH assay), hydroxyl radical scavenger activity, superoxide radical-scavenger activity and lipid peroxidation inhibition are commonly used to determine the antioxidant activity of mango fruit.

Table 2.3 Physicochemical, bioactive compounds and antioxidant capacity of ‘Nam Dok Mai Si Thong’ mango fruit

Characteristics	Value
TSS (°Brix)	20.1
TA (%)	0.3
Sugar content (g/100g DW)	Value
Fructose	19.47
Glucose	6.28
Sucrose	28.13
Vitamin C (g/100g)	3-5
Total carotenoid (mg/100g DW)	50-60
Total flavonoids (mg/100g DW)	60-70
Total phenolics (mg/100g DW)	20
Antioxidant capacity (μM/g DW) [DPPH method]	50-60

Note DW – dry weight

Source Romainum et al. (2018)

2.5 Anti-inflammatory Properties

Inflammation is a part of the defense mechanism in the body. The immune system detects the inflammation and then removes harmful and external stimuli and

starts the healing process. Inflammation can be acute or chronic in character (Michels da Silva et al., 2019; Zhang et al., 2019). Acute inflammation defined as the tissue damage caused by microbial invasion, trauma, or toxic chemicals. The mechanism of acute inflammation begins quickly, has immediate effects, and the symptoms can be repaired. This effect can cause swelling, bumps, warmth, bruising, stiffness, and lack of mobility, as well as redness, discomfort, and tenderness. Chronic inflammation is defined as a slow and long-term effect that is present for several months to years. Oxidative stress and mitochondrial dysfunction such as increased formation of free radical molecules, uric acid crystals, advanced glycation end products and oxidized lipoproteins can be caused by inflammation and biochemical inducers (Pahwa et al., 2021).

2.5.1 Nitric Oxide Radical Scavenging Activity

NO is a diffusible free radical that functions as an effector molecule in a variety of biological processes in the human body. It has functions such as neuronal messenger, vasodilation, and antitumor and antimicrobial properties (Bhaskar & Balakrishnan, 2009). However, chronic exposure to nitric oxide radical is related with numerous carcinomas and inflammatory situations such as juvenile diabetes, arthritis, and ulcerative colitis. When NO interacts with the superoxide radical, its toxicity rises dramatically (Amaeze et al., 2011). Nowadays, natural products with anti-inflammatory activities have great interest. Oluwole and Esume (2015) found that acute inflammation was reduced by mango extract in a dose-dependent and significant way compared to controls. This suggests that mango extract contains active compounds with anti-inflammatory potential. Moreover, mangos have high phytochemicals such as flavonoids and polyphenolic compounds, and these nutrients promote anti-inflammatory activity (Cade, 2005; Yuan et al., 2006). The application of high hydrostatic pressure treatments (350 MPa and 600 MPa) improved the NO radical scavenging activity of pear peel (Gómez-Maqueo et al., 2019). Salicylic acid and methyl jasmonate effectively enhanced the nitric oxide inhibition in *Centella asiatica* (L.) urban leaves as used by medical herbs (Buraphaka & Putalun, 2020).

2.5.2 Hyaluronidase Enzyme Activity

Numerous physiological and pathological processes, including inflammation, have been linked to the hyaluronidase enzyme (Girish & Kemparaju, 2007). For the body to maintain proper homeostasis, the regulation of hyaluronidase by adequate inhibitors will be helpful. In order to identify substances having anti-inflammatory activity, it would be helpful to identify and characterize hyaluronidase inhibitors. In fact, numerous studies demonstrate that the ability of various chemicals found in plant-derived products to reduce inflammation is measured by the inhibition of hyaluronidase activity. According to research by Bralley et al. (2008), sorghum bran extracts with high total phenolic and antioxidant (FRAP) values block hyaluronidase *in vitro* more efficiently than extracts with lower phenolic contents of sorghum bran, wheat bran, or rice bran. The treatment with high pressure processing (HPP) resulted in a significant improvement in the inhibition of hyaluronidase in the treated onion in comparison with the untreated onion (González-Peña et al., 2013).

2.6 Quality Loss

Mangoes are climacteric fruits, meaning they produce a large amount of ethylene and have a rapid rate of respiration during the postharvest stage. The huge release of ethylene in climacteric fruits happens at the respiratory peak time, resulting in the most suitable ripening (Giovannoni, 2004). Climacteric fruits ripen more quickly and are characterized by rapid senescence, loss of sensory qualities, and lower nutritional quality, making edible tissues more susceptible to pathogens. Furthermore, the ripening process comprises a series of biochemical, physiological, and structural changes (Prabha & Bhagyalakshmi, 1998). Carbohydrates play a crucial part in the ripening process by way of depolymerization, leading to lower molecular size with a concurrent increase in the levels of ripening that activate certain enzymes, whose targets change from fruit to fruit. Starch, pectin, cellulose, and hemicelluloses are the four main groups of cell wall polysaccharides that undergo modifications during ripening. The primary cell wall and middle lamella of fruits include pectin, which are common and important components. Pectin also improve the texture and quality of

fruits. Their degradation during ripening seems to be responsible for tissue softening of a number of fruits. Excessive textural softening causes negative effects or spoilage during storage (Prasanna et al., 2007). Mango fruit ripening can affect the nutritional status of mangos. Macro nutrients such as carbohydrates, lipids and fatty acids, protein and amino acids, and organic acids and micronutrients (vitamins, minerals), and some non-nutrients (polyphenols and plant pigments) can be affected during mango fruit ripening. In addition, the rapid rate of ripening in fresh mango fruits can be attributed to disease infection, physical and chemical composition during the postharvest supply chain (Singh, et al., 2013b).

Senescence has great impacts on fruit postharvest quality and resistance to pathogen attack and environmental stress (Tian et al., 2004). In general, fungal pathogens can easily attack senescent fruit, and the diseases that cause can significantly increase fruit senescence after harvest (Tian et al., 2007). The oxidative stress causes ROS and the accumulation of ROS can result in oxidative damage to mitochondrial proteins, which can then lead to dysfunction of various mitochondrial components and finally speed up aging (Chan, 2006). When fungal infections attack fruits, they frequently run into host defense mechanisms, such as the buildup of chemicals that form barriers and the development of antimicrobial molecules that directly prevent pathogen invasion (Tian et al., 2006). One of the first host responses to a pathogen attack is an oxidative burst, in which numerous host enzyme systems of a plant produce huge amounts of ROS (Mellersh et al., 2002). Cell wall/membrane integrity, cell homeostasis, metabolism of reactive oxygen species generating oxidative burst, and membrane lipid peroxidation are all factors that influence fruit quality (Tian et al., 2010).

Preharvest environmental factors such as light, temperature, water, and carbon availability also influence the mango fruit postharvest quality. During preharvest condition, photosynthetic adaptation to light is primarily influenced by the mass-to-area ratio, with changes in the distribution of total leaf nitrogen playing a smaller effect at low irradiance (Urban et al., 2003). Fruit growth in terms of dry mass is decreased if the carbon supply to the leaf diminishes. Moreover, the level of carbohydrates received by mango affects the size of the mango fruit. Chemical residues from the usage of fungicides and plant growth stimulators can have an

influence on human health and the environment. In this phenomenon, potent alternative safety techniques are required to withstand environmental conditions by increasing nutritional properties and defense mechanisms. This preharvest condition of mango fruit is essential for postharvest performance of produce (Léchaudel & Joas, 2007). To manage postharvest ripening and environmental condition, an optimal storage temperature is supplied. On the other hand, new techniques for postharvest maintenance technology are still needed (Zeng et al., 2006).

2.8 Pre-harvest Treatments

Many technologies are now being used to maintain the postharvest quality of mango fruits. Nowadays, pre-harvest condition of mango is also considered to preserve the quality of mango fruits. One month prior to harvest, bagging of fruit are used to protect them from insect and physical damage with newspaper or brown bags. Moreover, a good water supply is very important to get high quality fruit. The time of fertilizer application, thinning and pruning is also important to improve mango fruit size and quality (Maqbool & Mazhar, 2007). Besides, pre-harvest treatments of mango are often applied by spraying with chemical substances. These chemical treatments are mostly used as growth regulators to slow down the ripening process and reduce disease infection and post-harvest quality. In a previous study, ethephon, gibberellic acid (GA_3), polyamines, and salicylic acid were used in the spraying approach to preserve postharvest quality (Lara Ayala, 2013).

2.9 Postharvest Treatments

Major postharvest issues with mango fruit quality are prevalent in tropical and subtropical developing regions where there aren't enough postharvest handling methods available or easy to apply (Ntsoane et al., 2019). Heat treatment, storage techniques, edible coatings and chemical treatments such as 1-methylcyclopropene and methyl jasmonate commonly used in postharvest treatments (Bambalele et al., 2021)

2.9.1 Heat Treatment

Heat treatments have become more popular over the past ten years in the fruit industry and are now used to reduce postharvest diseases due to their fungicidal and insecticidal properties and insect pests in many nations. However, depending on the treatment temperature, duration, commodities, cultivars, size or weight, and maturity stage of the fruit, it has been observed to have a negative impact on the fruit's quality (Anwar & Malik, 2007; Jacobi et al., 2001; Sivakumar et al., 2011). Mangoes are frequently subjected to the following heat treatments: forced hot air treatment, hot water treatment, and vapour heat treatment (Anwar & Malik, 2007). It has been demonstrated that the mango fruit sector can benefit from the hot water treatment method, which leads to less fruit loss and higher market value. It is advantageous for small- and medium-sized farmers and packing houses, is simple to implement, and is environmentally friendly (Sivakumar et al., 2011). In order to make sure that the heat from the heating medium, often hot air or hot water, gets transferred to the fruit, it is heated for a set amount of time (Jha et al., 2010). Heat-induced fruit ripening is related to an increase in carotenoid biosynthesis, chlorophyll breakdown, and the production of cell wall degrading enzymes (Jacobi et al., 2001; Jha et al., 2010). Heat treatment has been demonstrated to improve fruit quality by inhibiting chilling injury, controlling insects, diseases, and decomposition, and delaying the ripening process (Tian et al., 2010). Temperature recommendations range from 43-46 °C for 65-90 min, depending on fruit size and cultivar. Extreme hot water temperatures above 46 °C, on the other hand, cause extensive damage (Jha et al., 2010).

2.9.2 Cold Storage

Low temperature is frequently used during storage to slow down fruit metabolism, delay ripening and senescence, reduce water loss, prevent or reduce disease and insect activity, and increase postharvest shelf life (Sudhakar Rao & Gopalakrishna Rao, 2008). On the impact of low temperatures on the mango fruit's quality, extensive research has been done. Depending on the cultivar and fruit maturity, a recommended storage temperature range of 5–15 °C for a period of 2–3 weeks was indicated (Singh et al., 2012; Singh, et al., 2013b; Watanawan et al., 2014). Some cultivars at various stages of maturation that are exposed for a specific

amount of time to extremely cold temperatures below 10 °C develop chilling injury (Singh et al., 2012). In the fruit sector, keeping temperatures low is crucial. In the supply chain for exports, low temperature storage for two to three weeks is insufficient. To extend the shelf life of mangoes, a combination of other strategies is necessary (Ntsoane et al., 2019).

2.9.3 Edible Coatings

Both developed and developing countries use edible coatings to increase the mango fruit's shelf life. Application of edible coatings is a desirable, eco-friendly postharvest process that can extend shelf life, slow water loss, delay ripening, and maintain sensory qualities while increasing the amount of soluble solids, titratable acidity, and ascorbic acid and preventing the growth of microorganisms. It functions as a water and gas exchange rate (O_2 and CO_2) barrier by forming a transparent layer on the fruit's surface, which alters the composition of the fruit's interior atmosphere (Singh, et al., 2013b). The effectiveness of various edible coatings is influenced by temperature, relative humidity, composition, and genotype (Kittur et al., 2001). Mangoes with chitosan coatings have longer shelf life, more total carotenoids, and more vitamin C (Gonzalez-Aguilar et al., 2010; Kittur et al., 2001).

2.9.4 Methyl Jasmonate and 1-Methylcyclopropene

The fruit sector is slowly adopting postharvest methyl jasmonate application for quality maintenance, including induction of plant defense mechanisms to defend disease and stimulation of changes in the fruit's physical appearance, mechanical characteristics, and bioactive compounds (Boonyaritthongchai et al., 2016; González-Aguilar, Fortiz, et al., 2000; Lalel et al., 2003). It has been reported that using 10^{-5} M methyl jasmonate treatments as a postharvest treatment significantly lowered fruit deterioration, decay, firmness, improve color, and chilling injury symptoms while maintaining total soluble sugars and organic acid levels in cv. 'Kent' (González-Aguilar, Wang, et al., 2000).

1-MCP, an ethylene antagonist, is a gaseous substance that is readily available for commercial usage, nontoxic, and effective at inhibiting the activity of ethylene at very low doses (Vázquez-Celestino et al., 2016). 1-MCP treatment can be applied to both domestic and imported mangoes (Ngamchuachit et al., 2014). In the 'Kensington

Pride' mango, 1-MCP treatment preserved fruit firmness while lowering levels of citric acid, malic acid, succinic acid, total organic acids, total sugars, and sucrose. However, it decreased the accumulation of total sugars and sucrose (Razzaq et al., 2016). This may be caused by enzymatic hydrolysis changes in the cell walls during ripening (Tharanathan et al., 2006) and a delayed conversion of starch to sugars, which contributes to fruit sweetness (Razzaq et al., 2016).

2.10 Salicylic Acid

Fruit quality changes during ripening and senescence include softening, a decrease in total acidity, an increase in sugar content, the development of color and aroma, among other factors (Asghari & Aghdam, 2010). Ethylene plays a key role in fruit ripening and senescence. Fruit ripening and senescence are significantly influenced by ethylene (Fischer & Bennett, 1991). SA, also known as ortho-hydroxybenzoic acid, is an endogenous signaling molecule that regulates stress responses and plant physiological activities such as photosynthesis, heat production, stomatal conductance, transpiration, disease resistance, seed germination, and glycolysis (Klessig & Malamy, 1994). Exogenous SA treatment significantly reduces ethylene production in a variety of horticultural crops, thus extending shelf life.

In various horticultural crops, increased firmness retention as a result of SA treatment has also been observed. According to Srivastava and Dwivedi (2000), SA improved firmness retention during storage and delayed ripening in banana fruit by inhibiting ethylene biosynthesis or its effects. Applying SA lowers ethylene synthesis, which in turn slows the activity of enzymes that break down cell walls and membranes, including polygalacturonase, lipoxygenase, cellulase, and pectinmethylesterase. This results in a slower rate of fruit softening (Asghari & Aghdam, 2010; Zhang et al., 2003).

TSS concentration increases during fruit ripening as a result of the action of sucrose-phosphate synthase, an ethylene stimulated enzyme. TSS is a key component in the ripening of horticultural crops, particularly fresh fruits (Asghari & Aghdam, 2010). Cell walls contain large amounts of polysaccharides, primarily pectins and cellulose, which cause a rise in TSS content when affected by the activity of cell wall

degrading enzymes during ripening. SA treatment protects cell walls by reducing these enzyme activities, thereby effectively preventing the increase in TSS content. SA treatment protects cell walls and effectively prevents the increase in TSS content by lowering these enzyme activities.

Several studies show that SA has direct antifungal effects on pathogens. In strawberry fruit, exogenous SA treatment (1-2 mmol L⁻¹) significantly decreased fungal decay. SA was applied both pre- and postharvest to effectively reduce deterioration and extend the shelf life of strawberries (Babalar et al., 2007). The concentration of 2 mM SA also reduced the disease incidence in 'Chausa' mango (Barman & Asrey, 2014). When SA was applied to pear fruit by dipping the fruits in SA solution (1 mmol L⁻¹), postharvest deterioration during cold storage was considerably minimized (Asghari et al., 2007).

SA is a signaling molecule and exhibits elicitor activity and improves bioactive metabolites in plants (Horváth et al., 2007). Bioactive compounds like carotenoid, vitamins, and total phenolics have the potential to improve the nutritional value of fruits and vegetables while also increasing their resistance to biotic and abiotic stress (Coste et al., 2011; Smetanska, 2008). Preharvest SA applications have also maintained TPC and total antioxidant activity of tomatoes (Baek et al., 2021). In addition, preharvest applications of SA maintained postharvest quality by enhancing the preservation of SSC, TA, and ascorbic acid and total antioxidant contents of 'Amrapali' mango fruit (Reddy & Sharma, 2016). Abbasi et al. (2020) stated that exogenous application of salicylic acid and gibberellic acid enhanced anti-inflammatory secondary metabolites production in multiple shoot culture of *Ajuga integrifolia* Buch. Ham. ex D.Don. Moreover, salicylic acid increased the anti-inflammatory activity of aloe vera adventitious root (Lee et al., 2013).

2.11 Nitric Oxide

NO is a hydrophobic, highly diffusible gaseous molecule that regulates a wide range of plant growth and developmental processes, including germination, metabolism, signal transmission, and senescence (Besson-Bard et al., 2008).

Endogenous NO levels were found to be higher in unripe climacteric and non-climacteric fruit (Singh, Khan, et al., 2013). NO has a volatile nature and is a highly diffusible free radical, which causes reactive oxygen species toxicity. However, it can be used as an anti-ethylene agent to maintain the ripening process and shelf life (Pareek, 2017).

A physiologically mature fruit changes from an undesirable to an advanced level of color, softness, scent, taste, and flavor with maximal consumer acceptance during ripening, a distinctive and complex biochemical process (Pareek, 2017). However, as fruit ripens, starch is considerably converted into simple sugars, total and individual phenolic levels decrease, the biosynthesis of volatile aromatic compounds increases, and chlorophyll is substantially degraded in favor of the synthesis of pigments (Clendennen & May, 1997; Kader, 1997). Reduced ethylene production and/or its effects may be related to NO's antiripening, fruit softening, and senescence properties (Leshem & Pinchasov, 2000). SNP, NO-donor, significantly delayed ripening in apple fruit during ripening (Cheng et al., 2009).

Fruit softening is typically reduced by the depolymerization of certain polysaccharides and pectin components. The activities of several cell wall hydrolysis enzymes such as polygalacturonase (PG) and pectin esterase (PE) regulate fruit softness during ripening (Ali et al., 2004; Khan & Singh, 2007; Khan et al., 2007). During cold storage, NO application decreased the activity of PG enzymes in peach fruit (Zhu et al., 2010). In a different study, PG and PE enzyme activity during ripening were lowered in banana fruit treated with NO (Cheng et al., 2009; Yang et al., 2010). NO treatment can successfully delay the ripening and softening of papaya fruit, probably through the regulation of enzymes and hormones associated to cell wall softening. Papaya fruit treated with NO had higher firmness during storage and decreased in activities of PG, PE, and cellulose (Guo et al., 2003).

The nutritional quality of fruits and vegetables, particularly their protective role against many chronic oxidative stress-related diseases, cancer, and aging-related disorders (Huang et al., 2005), has also increased consumer interest in purchasing more nutritious fruit crops. NO decreased cell membrane disintegration, resulting in less electrolyte leakage and better preservation of certain cellular pigments, TSS, TA, and antioxidant chemicals, primarily ascorbic acid (Flores et al., 2008). Postharvest

application of SNP promoted TPC and preserved antioxidant properties in ‘Tainong’ mango fruit (Ren et al., 2017). NO can increase the activity of defense-related enzymes (Hu et al., 2014). Pre-harvests SNP spray reduced fruit decay and stimulated TPC and flavonoids in muskmelon fruit (Wang et al., 2019).



CHAPTER 3

RESEARCH METHODOLOGY

3.1 Materials

3.1.1 Plant Material

Freshly harvested ‘Nam Dok Mai Si Thong’ mangoes with 80-90% maturity stage were used as a raw material which were purchased from a commercial orchard in Chiang Rai Province, Thailand.

3.1.2 Chemicals

Hexane, gallic acid, Folin-Ciocalteu phenol's reagent, sodium carbonate, Trolox, DPPH, methanol, sodium acetate, acetic glacial, hydrogen chloride, 2,2-diphenyl-1-picrylhydrazyl, 2,4,6-Tris (2,4-pyridyl)-s-triazine solution, ferrous sulfate, iron chloride sulfanilamide, naphthylethylenediamine, sodium nitroprusside, sodium chloride, sodium hyaluronidase, hyaluronidase, hexadecyltrimethylammonium bromide, sodium hydroxide.

3.2 Salicylic Acid and Sodium Nitroprusside Application

3.2.1 Pre - And Post-harvest Application of SA

Eight-year-old mango trees from an orchard in the Mae Kon Sub District, Chiang Rai Province, Thailand were selected for this study. Approximately 100 mango fruit, formed approximately 80 days after flowering, were chosen based on their size and lack of physical damage. These mango fruits were randomly selected from different parts of each tree. The fruits were divided into four groups (25 fruits in each group) based on their applications as shown in Table 3.1. For preharvest (Pre SA) and postharvest (Post SA) SA treatments and their combination

(Pre + Post SA), aliquots of 25 fruit were treated. For the Pre SA treatment, fruit were sprayed with 2 mM SA using a hand-sprayer until the solution was thoroughly applied to the entire fruit. Three weeks after the Pre SA treatment, the fruit were harvested and immediately transferred to the postharvest laboratory within 1 h.

At the postharvest laboratory, the fruit maturity was checked with 2% sodium chloride (NaCl) solution and then selected 200 fruits for 25 fruits of each treatment with the same maturity and free from disease infection. These 200 fruits were cleaned with 200 ppm sodium hypochlorite (NaClO) and immersed in hot water (49°C, 10 minutes) to ensure they are free of disease infection. For the Post SA treatment, the fruit were dipped for 10 min in 2 mM SA solution immediately after being immersed in the hot water. Then, all fruits were dried at room temperature, and then stored at 13 °C with 80–85% relative humidity; one aliquot of fruit was sprayed and dipped with water to act as control. The fruits were sampled every 5 days until the 20th day of storage. Process of pre- and postharvest SA treatments on ‘Nam Dok Mai Si Thong’ mango fruit is seen in Figure 3.1.

Table 3.1 Pre- and postharvest SA treatments applied to ‘Nam Dok Mai Si Thong’ mango fruit

		Treatment			
		Control	Pre SA	Post SA	Pre SA + Post SA
Preharvest spray	SA (2.0 mM)		+		+
	Water	+			
Postharvest immersion	SA (2.0 mM)			+	+
	Water	+			

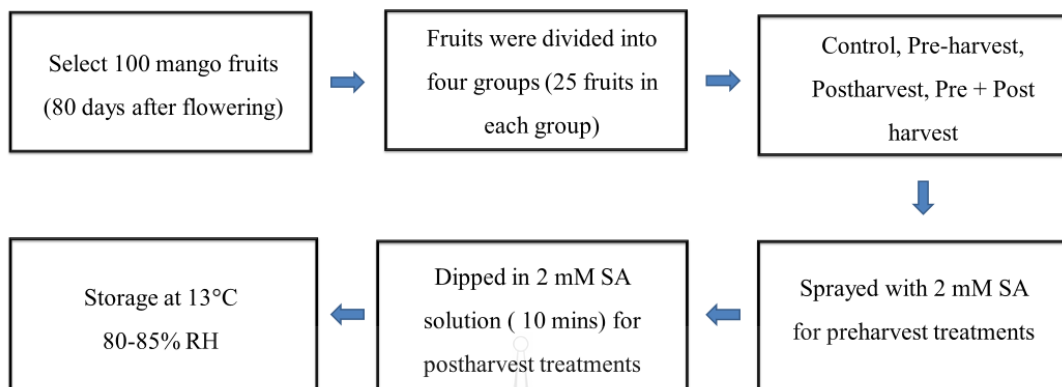


Figure 3.1 Process of pre- and postharvest SA treatments on ‘Nam Dok Mai Si Thong’ mango fruit

3.2.2 Pre - and Postharvest Application of SNP

SNP pre- and postharvest application procedures were the same as SA pre- and postharvest application methods. SNP treatment for pre-harvest application took place on the same day as SA treatment for pre-harvest and postharvest application. However, in order to focus more on quality analysis, the quality assessment of SNP sample fruit began the next day after SA sample fruit. Process of pre- and postharvest SNP treatments on ‘Nam Dok Mai Si Thong’ mango fruit is shown in Figure 3.2.

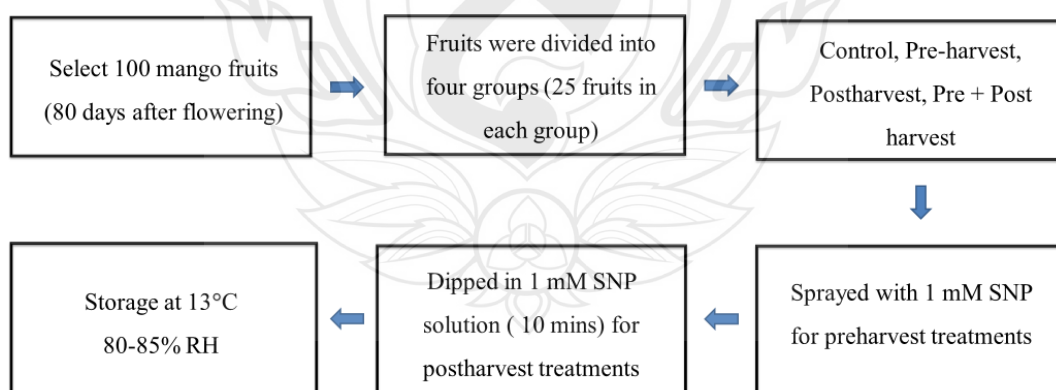


Figure 3.2 Process of pre- and postharvest SNP treatments on ‘Nam Dok Mai Si Thong’ mango fruit

3.3 Decay and Physio-chemical Quality Analysis

3.3.1 Fruit Decay (%)

The percentage of decayed fruit was calculated by counting the number of fruit decay out of the total.

$$\text{Fruit decay (\%)} = \frac{\text{the number of decay incidence fruit}}{\text{the total number of fruits}} \times 100$$

3.3.2 Weight Loss (%)

Weight loss was measured every 5 days using the initial weight. Five fruit from each treatment were selected from whole fruit and used as a non-destructive approach in this study. The percentage of weight loss was calculated according to the following equation.

$$\text{Weight loss (\%)} = \frac{\text{Initial Weight (g)} - \text{Final Weight (g)}}{\text{Initial Weight (g)}} \times 100$$

3.3.3 Color Measurement

The color was measured 2 times per one fruit on the middle of mango peel by colorimeter (CM-600d, Konica Minolta, Tokyo, Japan) and expressed by the values of L *, a *, b *, hue angle and chroma. These values were then used to calculate total color differences (ΔE) (Chen & Ramaswamy, 2002) using the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$$

3.3.4 Fruit Firmness

The texture analyzer (TA-XT plus, stable micro system Co., Ltd., Surrey, UK) was used to determine fruit firmness, and results were reported in Newtons (N). As a destructive approach, 5 fruit per treatment are selected before analysis. A pressure test of p/6 (6 mm) probe was used, with a depth of 10 mm twice for each fruit.

3.3.5 Soluble Solid Content (SSC) and Acidity (%)

SSC was analyzed using a digital refractometer (Model: PR-a, ATAGO, Tokyo, Japan). Extracted solution of sample fruit 1g was combined with 49 g of distilled water and then tested with a total acidity meter (Model: PAL-Easy ACID F5, ATAGO, Japan) to determine the acidity (%).

3.4 Bioactive Compounds

3.4.1 Carotenoid Content

The total carotenoid was calculated following the method of Desobry et al. (1997) with some adjustments. 2.5 mL distilled water and 25 mL of 95 percent hexane was combined with five gram of sample. The mixture was then homogenized for one minute before being centrifuged for ten minutes at $5000 \times g$, 4°C . Following that, a spectrophotometer (Model: GENESYS 10S, Thermo Scientific, Waltham, MA, USA) was used to detect the sample's absorbance at 454 nm. The total carotenoid content of mango was calculated based on the absorption of the carotenoid in the solvent used and expressed as $\mu\text{g g}^{-1}$ dry weight (DW).

3.4.2 Sample Extraction Of TPC And Other Biochemical Analysis

This extraction was followed the method of Sogi et al. (2012). Briefly, fresh pulp (1 g) were mixed with 20 mL of 95% methanol, kept in a water bath shaker at 20°C for 1 h, and then centrifuged at $10,000 \times g$ for 10 min. The supernatant was collected and residues were re-extracted using 10 mL of 95% methanol by vortexing for 1 min, followed by centrifugation at $10,000 \times g$ for 10 min. For final analysis, both supernatants were combined.

3.4.3 Total Phenolic Content

The total phenolic content was measured using gallic acid as a standard, as defined in ISO 145021 (2005). A 0.25 mL of sample was added in the test tube with 1.25 mL of 10% Folin-Ciocalteu phenol's reagent and 7.5% w/v sodium carbonate 1 mL was added. After one hour under normal temperature, the combination was tested for absorbance at 756 nm using a spectrophotometer with water as a blank. The standard curve was calculated with gallic acid equivalence (GAE) and the total phenolic was showed as mg GAE g^{-1} DW.

3.5 Antioxidant Capacity

3.5.1 DPPH Radical Scavenging Method

The free radical scavenging activity is determined using a modified Molyneux (2004) technique with slight modification. The 50 μL of crude extract was combined with 1.95 mL of DPPH-methanolic solution. The mixture was then kept in the dark for 30 minutes at room temperature (25 $^{\circ}\text{C}$), and the absorbance was assessed at 517 nm. The amount of DPPH radical scavenging activity per gram of dry weight sample was measured in micromoles of Trolox equivalent (TE) per gram of dry weight sample ($\mu\text{M TE g}^{-1}\text{ DW}$).

3.5.2 Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power was evaluated according to the methodology of Benzie and Strain (1996) with modifications. The crude extract 400 μL was mixed into 2.6 mL of FRAP reagent [consisting of acetate buffer, 2,4,6-Tris (2,4-pyridyl)-s-triazine (TPTZ) solution, and ferric chloride in a 10:1:1 ratio]. Then, the solution was incubated at 37 $^{\circ}\text{C}$ for 30 min and measured at an absorbance of 595 nm. The FRAP content was expressed as $\text{mM FeSO}_4 \text{ g}^{-1}\text{ DW}$.

3.6 Anti-inflammatory Activity

3.6.1 Nitric Oxide Radical Scavenging Activity

This activity was analyzed according to the method described by Hazra et al. (2008). A 40 μL of diluted extract was mixed with 800 μL of 10 mM sodium nitroprusside. The reaction solution was then incubated for 2h and 30 min at room temperature. Then, 200 μL of reactive solution was moved to a new test tube after incubation, and 400 μL of 0.33 % sulfanilamide in 20 % glacial acetic acid was added. The sample tubes were kept at room temperature for 5 min. 400 μL of 0.1 % N-(1-Naphthyl) ethylene-diamine dihydrochloride was added to the test tube and then stored at 25 $^{\circ}\text{C}$ for 30 min. The mixture was analyzed at 540 nm using a spectrophotometric cell reader. The concentration of the standard solution, ranging

from 0.2 to 0.5 mg mL⁻¹, was calculated by Trolox. The results were represented in millimoles of TE per gram of dry weight (mM TE g⁻¹ DW).

3.6.2 Hyaluronidase Inhibition Activity

The hyaluronidase inhibitory activity was performed following the method described by Bralley et al. (2007) with slight modifications. The mesocarp tissues (1g) was extracted in 50% ethanol (9 mL) and then stirred for 1 h at room temperature. The mixture was centrifuged at 1500 rpm for 10 min at 4°C and the supernatant was collected. The crude extract sample (15 µL) was mixed with 147 µL of 0.2 M sodium acetate (pH 6) and 120 µL of 0.5 mg/mL sodium hyaluronate, and the mixture was well stirred for 30 s. Then, the mixture was added 18 mL of 1 mg/mL hyaluronidase and then at 37°C for 15 min. The reaction mixture was stopped by adding 1.2 mL of 2.3 % hexadecyltrimethyl ammonium bromide (CTAB) in 2% NaCl (pH 12). At room temperature, the sample was incubated for 10 min. The hyaluronidase inhibitory activity was measured at 400 nm and expressed as the percentage (%) of inhibition.

3.7 Statistical Analysis

Treatments were conducted with five replications in a completely randomized design (CRD). The Statistical Analysis System (SPSS, version 25) was used to express the data using the mean and standard deviation for the analysis of variance (ANOVA). Tukey's multiple range tests were used to determine the treatment on dependent variables ($p < 0.05$).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Pre- and Post-harvest Application of Salicylic Acid

4.1.1 Decay and Physio-Chemical Quality in Mango Fruit

The visual appearance of the mango fruit can be seen in Figure 4.1. Control fruit showed decay at the end of the storage period. Fruit that received the Pre + Post treatment maintained good appearance compared to the control and other treated fruits. Fruit decay is one of the most important parameters affecting postharvest shelf life. No fungal infection was observed on the fruit during the first 5 days of storage (Figure 4.2 A). After that, the control fruit quickly decayed, and the percentage of decayed fruit was higher than that of all the SA-treated fruit. There were no significant differences among the SA-treated fruit, with all treatments having around 20% decay on days 15 and 20. SA promotes the expression of pathogenesis-related genes, thereby conferring resistance to pathogens (Malamy & Klessig, 1992). The combination of pre- and postharvest SA treatments also had the most beneficial effects on storage life and fruit quality of tomato fruit, including reductions in decay (Baninaiem et al., 2016).

Weight loss increased with storage time and reached its highest value of $11.57 \pm 0.37\%$ in control fruit, but it was significantly ($p < 0.05$) lowered in fruit given the Post SA, Pre + Post SA, and Pre SA treatments being 6.26 ± 0.49 , 6.81 ± 0.93 , and $7.8 \pm 0.28\%$, respectively (Figure 4.2 B). However, there were no significant differences in weight loss among the SA treated fruit. The main cause of weight loss in fresh fruits and vegetables is the loss of water due to transpiration and respiration. Respiration uses oxygen to break down glucose and release carbon dioxide and water; such a process reduces the weight of the fresh produce (Xanthopoulos et al., 2017). SA applications reduce respiration rates in horticultural crops thereby reducing weight

loss (Asghari & Aghdam, 2010); treatment with 2 mM SA has been shown to reduce weight loss from ‘Chausa’ mango fruits (Barman & Asrey, 2014). The fruit treated with Pre + Post SA maintained their visual appearance at the end of the storage period better than the controls (Figure 4.1) as a result of reducing shriveling and wilting.

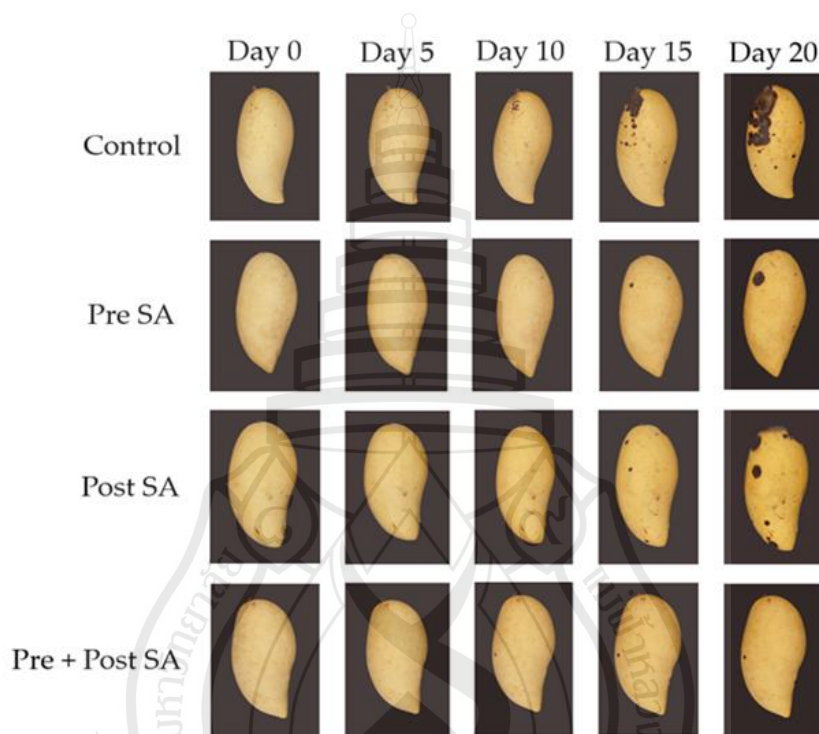
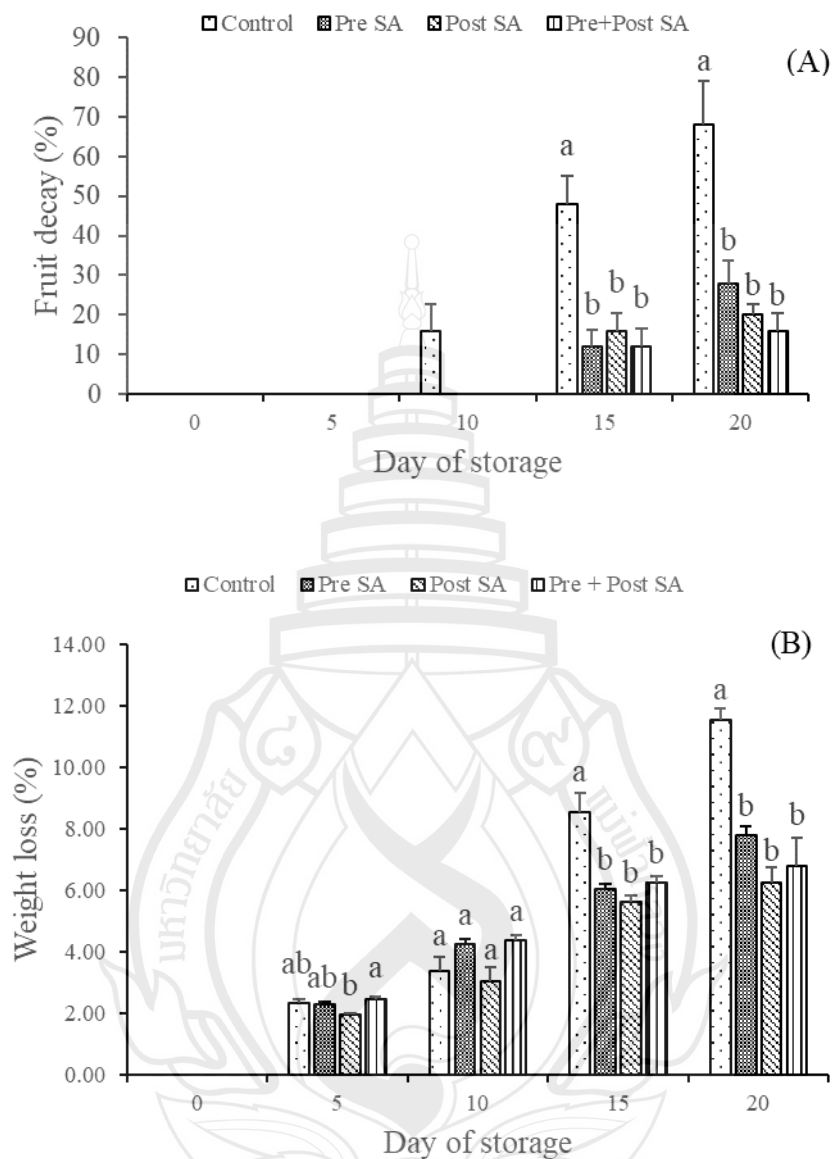


Figure 4.1 The visual appearance of control and SA-treated fruits during storage at 13°C for 20 days

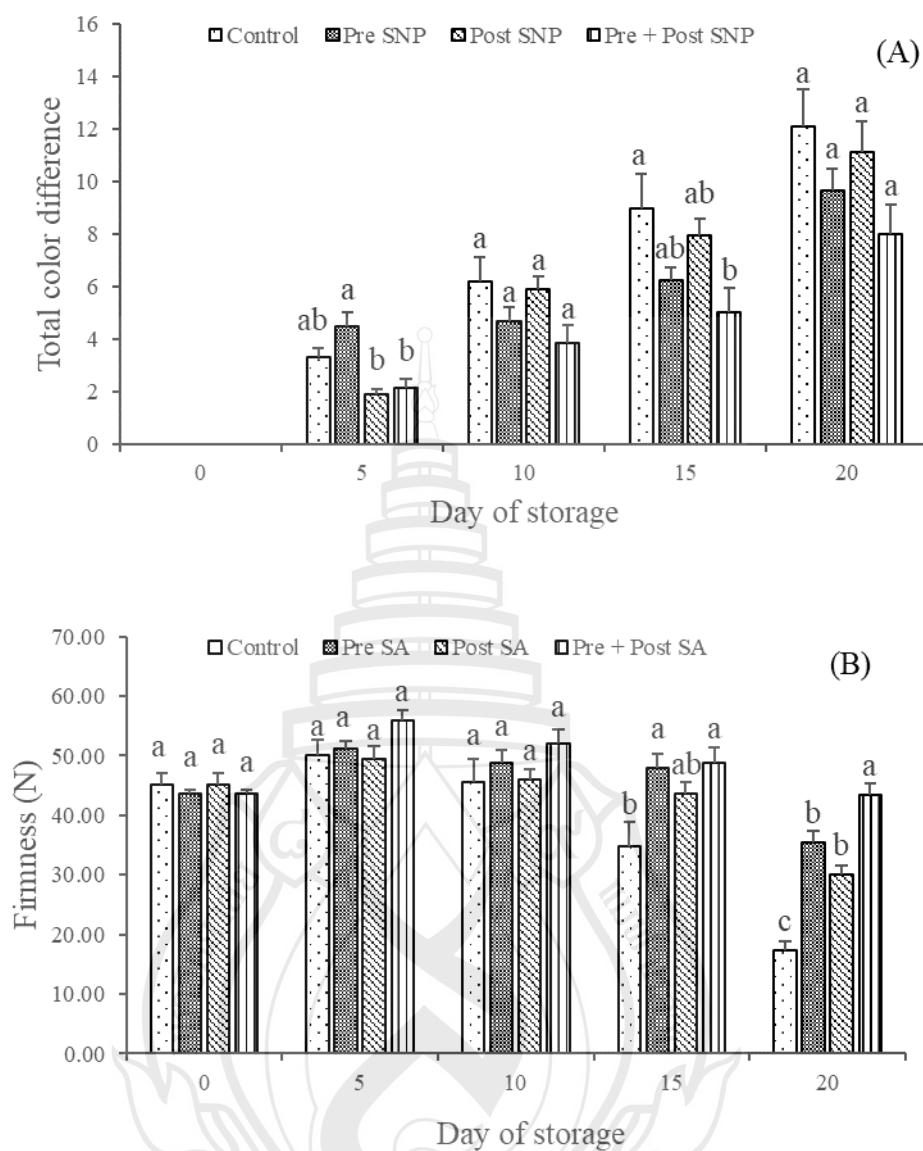


Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.2 Effect of SA application on (A) fruit decay and (B) weight loss of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

The total color difference (ΔE) of the fruits gradually increased during the storage period (Figure 4.3 A). There are two trends in the data. Firstly, the ΔE values of fruit in the control tended to be higher than those given the SA treatments. Secondly, fruit in the Pre + Post SA treatment tended to have the lowest ΔE values at each assessment time. The changes in peel color during the ripening process are due to a degradation of chlorophyll and the biosynthesis of other pigments (Jabbar et al., 2011), and treatment with SA appears to be slowing the ripening process and these changes in pigmentation. A similar effect of SA on color changes was found in sweet cherry (Valero et al., 2011) and peaches (Tareen et al., 2012).

Fruit firmness is one of the most important quality factors for consumer acceptance. The firmness of control fruit had increased slightly by day 5 (50.11 ± 2.48 N) but then decreased, particularly on days 15 and 20 (Figure 4.3 B). All treatments with SA maintained fruit firmness. Preharvest SA treatments have been shown to significantly retained firmness in lemons (Serna-Escolano et al., 2021) and 'Zil' mango (Hong et al., 2014). In our experiment, the Pre SA treatment also showed potential to maintain firmness of mango fruit. However, Pre + Post SA maintained the fruit firmness better than the other treatments. SA can beneficially maintain firmness by inhibiting cell wall and membrane degrading enzyme activities and by decreasing of the ethylene production (Srivastava & Dwivedi, 2000; Zhang et al., 2003).

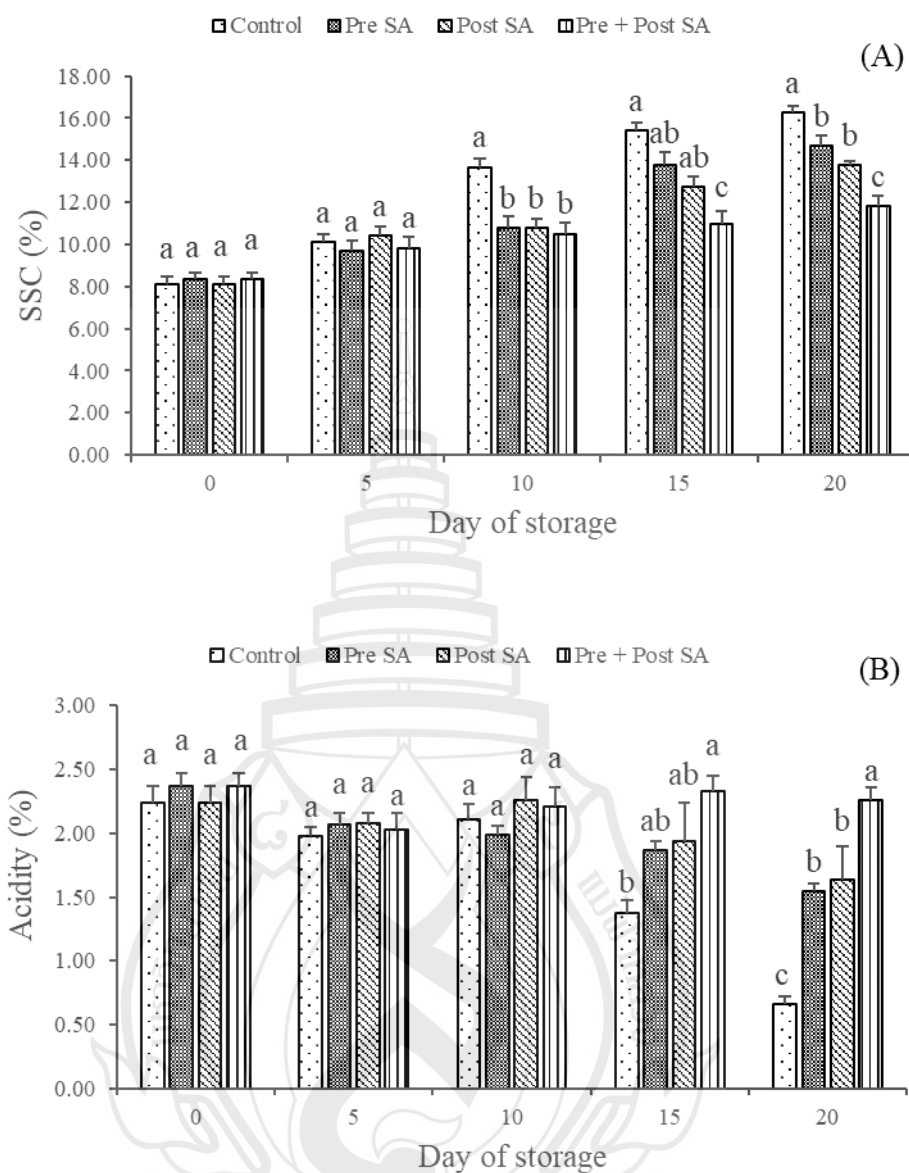


Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.3 Effect of SA application on (A) total color difference (B) firmness (N) of ‘Nam Dok Mai Si Thong’ mango fruit during storage at 13 °C

Effect of SA application on changes in the soluble solids content (SSC) of the mango fruit is shown in Figure 4.4 A. The SSC gradually increased in both control and treated fruit. On day 10 and onwards, the SSC contents of SA-treated fruit were significantly lower ($p < 0.05$) compared to those of control fruit. At the end of the storage period, the SSC of fruit in the Pre + Post SA ($11.82 \pm 0.51\%$) were significantly ($p < 0.05$) lower than those in the Pre SA ($14.7 \pm 0.47\%$) and Post SA ($13.79 \pm 0.15\%$) treatments. The SSC of mangoes increases during ripening due to starch breakdown (Peroni et al., 2008) and the actions of sucrose-phosphate synthase (Castrillo et al., 1992), and the lower SSC in SA treated mango fruits could be related to its ability to reduce the activity of this enzyme (Baenas et al., 2014).

Figure 4.4 B shows the effect of SA on acidity. A significant difference ($p < 0.05$) between the control and Pre + Post SA can be seen on days 15 and 20. Moreover, on day 20, the SA of fruit given the Pre + Post SA treatment was also significantly ($p < 0.05$) higher than that of fruit in the other SA treatments. On day 20, the percent acidity of the control was $0.66 \pm 0.05\%$ while those of fruit given the Pre SA, Post SA, and Pre + Post SA were $1.55 \pm 0.06\%$, $1.64 \pm 0.26\%$, and $2.26 \pm 0.1\%$, respectively. The decrease in acidity during the storage period could be due to the use of organic acids as a substrate for respiration. Preharvest SA treatment reduced reductions in TA of 'Amrapali' mango (Reddy & Sharma, 2016), and a combination of pre- and postharvest SA treatments had a similar effect in tomato fruit (Baninaiem et al., 2016).



Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.4 Effect of SA application on (A) soluble solids content and (B) acidity of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

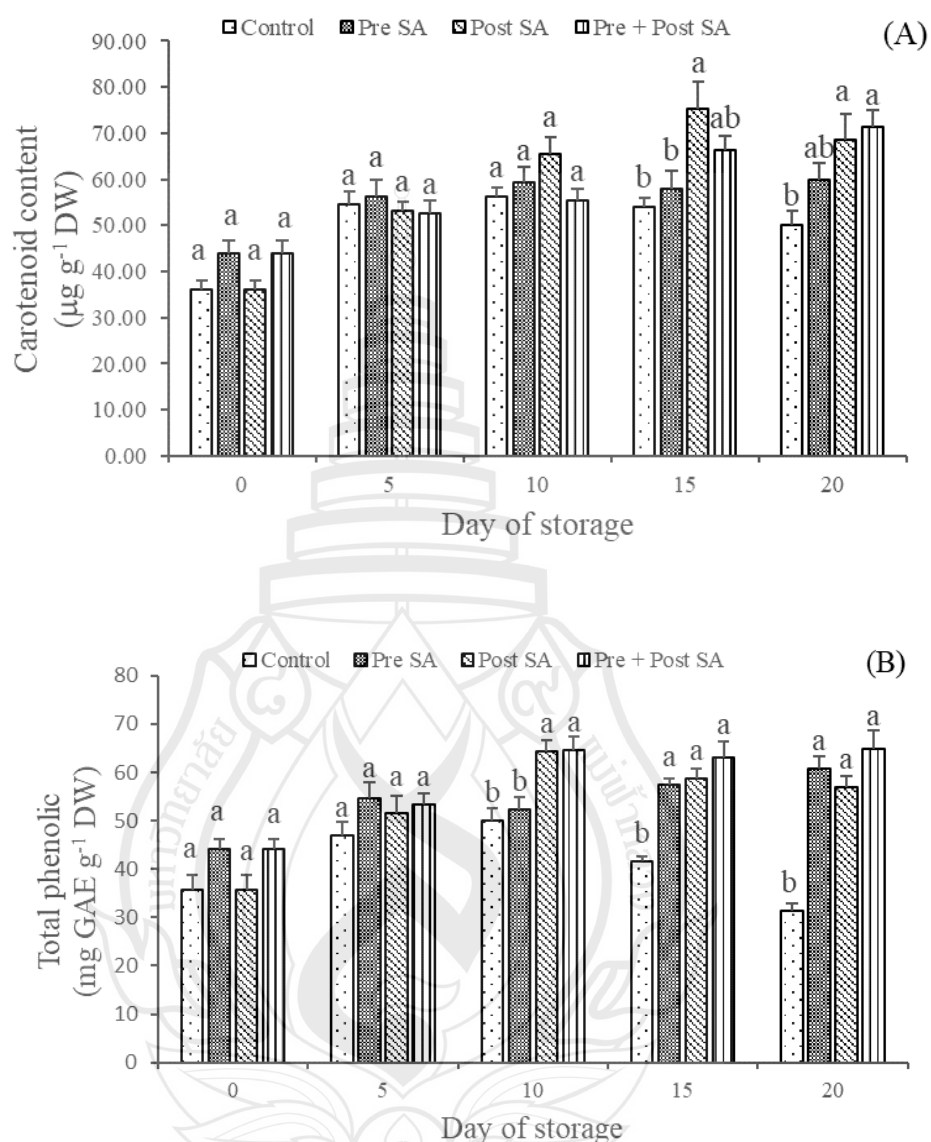
4.1.2 Bioactive Compounds

SA has been considered as an elicitor, as it increases concentrations of bioactive compounds such as phenolics, glucosinolates, carotenoids, betalains and vitamins that promote defense responses (Baenas et al., 2014). In this study, carotenoid and total phenolic contents were analyzed to determine the effects of SA on such bioactive compounds. Carotenoids can be considered as an important factor protecting organisms against photooxidative processes (Tapiero et al., 2004). The carotenoid content of the mango fruit gradually increased from day 0 to day 15 for all fruit (Figure 4.5 A). There was also a trend for fruit given a postharvest dip of SA to have higher carotenoid contents from day 15 onwards and there was no significant difference between the Post SA and Pre + Post SA fruits during the storage period. Postharvest applications of 2 mM SA have been shown to maintain carotenoid contents in ‘Chausa’ mango fruit (Barman & Asrey, 2014).

Phenolic compounds have antioxidant, anti-inflammatory, antimutagenic, antitumor, anticancer, antimicrobial, and cytotoxic properties. Phenolic compounds also have high antioxidant properties. Their structure is built around one or more aromatic rings bonded to at least one hydroxyl group. These hydroxyl groups are able to donate hydrogen atoms, which gives phenolic compounds their ability to neutralize free radicals, making them more stable and less toxic (Brito et al., 2020; Mahfuz et al., 2021). The effect of SA treatments on TPC can be seen in Figure 4.5 B. During the storage period, the TPC varied from 31.42 to 64.85 mg g⁻¹ DW. A significant difference ($p < 0.05$) between control and treated fruits occurred at days 15 and 20. There was no difference between SA-treated fruit in all storage periods; however, there was a trend for the fruit in the Pre + Post SA treatment to have the highest TPC. Beneficial effects of postharvest SA applications on TPC have been found for cornelian cherries (Dokhanieh et al., 2013) and papaya fruit (Hanif et al., 2020).

SA exhibit elicitor activity, and these elicitors are signaling agents that are important for growth and development as well as essential in the plant defense mechanisms against stress. Elicitation, particularly in respect to their health-promoting phytochemicals, has recently become popular as a method of enhancing the quality of edible plants. Treatments with elicitors could be a feasible way to trigger

the biosynthesis of bioactive metabolites (Meng et al., 2008; Pérez-Balibrea et al., 2011).



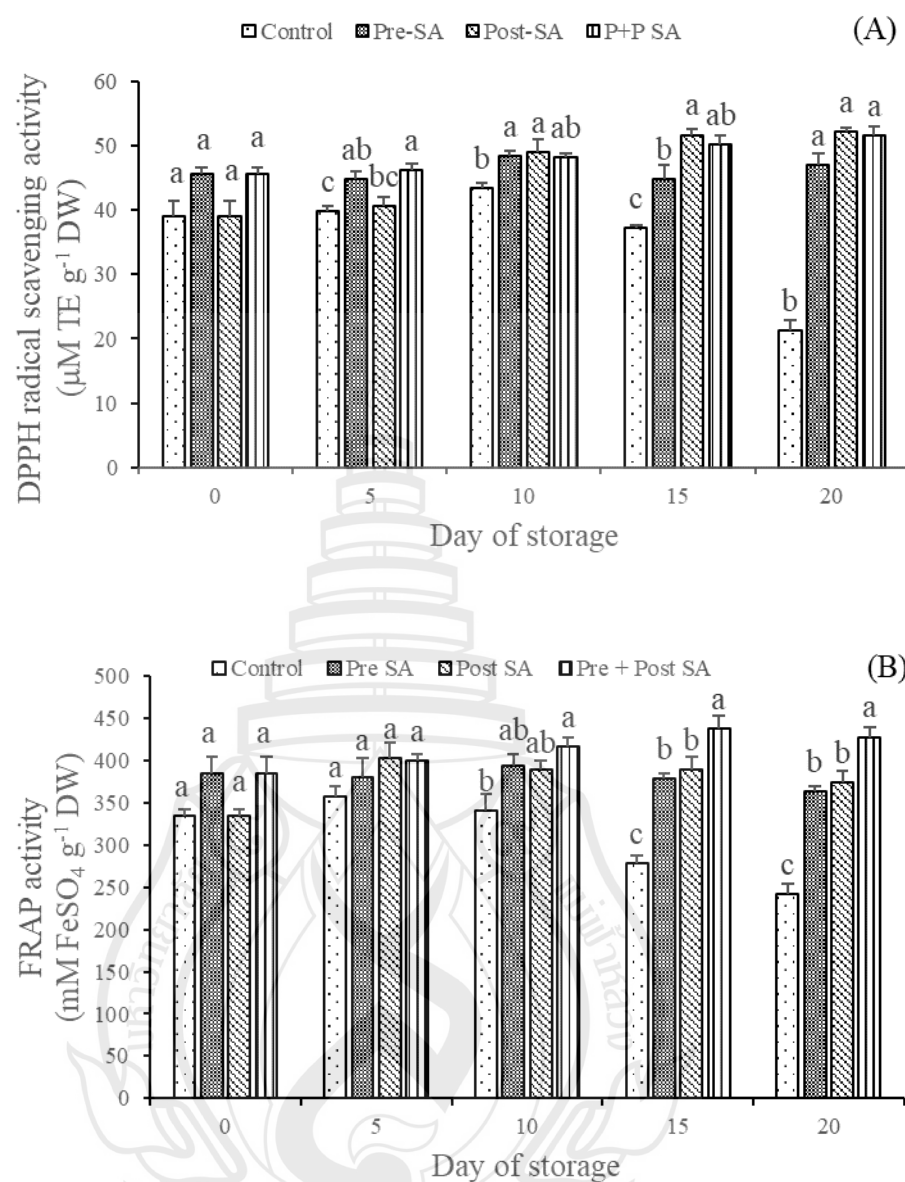
Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.5 Effect of SA application on (A) carotenoids and (B) total phenolic compounds of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

4.1.3 Antioxidant Activity

The DPPH radical scavenging activity (Figure 4.6A) of the control fruit gradually reduced after 10 days of storage, particularly after 20 days ($43.45 \pm 0.7 \mu\text{M g}^{-1} \text{DW}$ to $21.23 \pm 1.64 \mu\text{M g}^{-1} \text{DW}$). After 10 days of storage, the scavenging activity of all SA-treated fruit was significantly ($p < 0.05$) higher than that in control fruit. However, there were no significant differences among these treatments. Our results are in line with those of Dokhanieh et al. (2013) who reported that treatment of SA significantly increased the DPPH scavenging activity of ‘cornelian’ cherry fruits by enhancing concentrations of bioactive compounds like phenolics. This agrees with the results of present study that observed the increase of total phenolic content in SA treated mango fruits.

FRAP activity of all samples gradually increased up to day 15 but decreased slightly on day 20 (Figure 4.6B). A significant difference ($p < 0.05$) between control fruit and those in the Pre + Post SA treatment was found after day 10. At days 15 and 20, FRAP activity was significantly ($p < 0.05$) higher in fruit given the Pre + Post SA treatment than in the other two SA treatments. Erogul and Özsoydan (2020) showed that a preharvest application of 2 mM SA increased FRAP activity in the ‘Cresthaven’ peach.



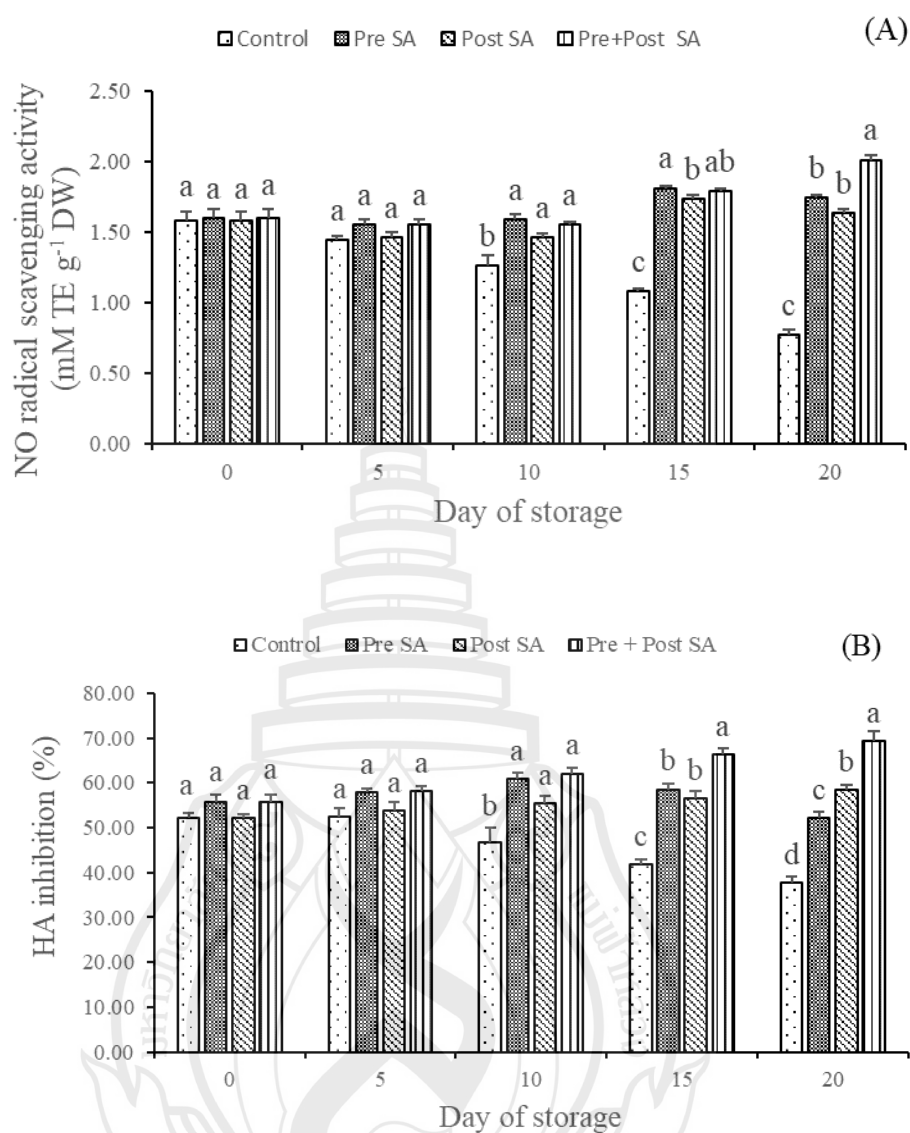
Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.6 Effect of SA application on (A) DPPH radical scavenging activity and (B) FRAP activity of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

4.1.3 Anti-inflammatory Activity

Exogenous application of SA increases anti-inflammatory activity and the production of secondary metabolites in *Ajuga integrifolia* Buch. Ham. ex D.Don, a plant grown as a source of medicine and for its essential oil (Abbasi et al., 2020) and in *Centella asiatica* (L.) Urban a plant also used as a medicinal herb as well as a culinary vegetable (Buraphaka & Putalun, 2020). In this study, SA treatments were applied to mango fruits to promote NO scavenging and hyaluronidase inhibition. The NO radical scavenging activity in fruit of the control treatment gradually decreased during storage (Figure 4.7A). A significant difference ($p < 0.05$) between control and all SA-treated mango fruit was observed after day 5. On day 20, activity in fruit of the Pre + Post SA treatment was significantly higher than those in other two SA treatments.

The effects of SA treatment on hyaluronidase inhibition activity are shown in Figure 4.7 B. No significance difference was seen between control and treated samples in the early storage period up to day 5. After day 5, the HA inhibition activity of control fruit remarkably declined rapidly, and a significant difference ($p < 0.05$) was found between the control and all SA-treated fruit. On days 15 and 20, HA inhibition was higher in fruit in Pre + Post SA treatment than those in the other two SA treatments. Inflammatory responses occur when tissues are injured or infected by microbes, leading to a complex process involving immune cells and pro-inflammatory mediators (Yun et al., 2008). SA is an elicitor produced in plants that mediate signal transmission in plant defense responses (Bulgakov et al., 2002), and in our study, SA maintained the anti-inflammatory properties of mango fruits. However, clarification of the mechanism by which SA on anti-inflammatory activity needs further study.



Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.7 Effect of SA application on (A) NO radical scavenging activity (mM TE g⁻¹) and (B) HA inhibition (%) of ‘Nam Dok Mai Si Thong’ mango fruit during storage at 13 °C

4.2 Pre- and Post-harvest Application of SNP

4.2.1 Decay and Physio-Chemical Quality in Mango Fruit

The physical appearance of the mango fruit has been expressed in Figure 4.8. The most severe decay can occur in control at the end of the storage period. Pre + Post SNP treatment showed a better appearance than the control, Pre SNP, and Post SNP treated fruits. Figure 4.9 A shows that percentage of decayed fruit increased during storage in all treatments from Day 10 onwards. In addition, treatment with SNP significantly reduced decay compared to the controls, and among the SNP-treated fruit, there was a trend for the combined treatment to have the greatest effect and the Pre SNP treatment the least; however, no statistically significant effect was found. Reductions in decay resulting from treatment with SNP have been found for litchi (Barman & Asrey, 2014) and 'Tainong' mango (Ren et al., 2017), and NO has been shown to induce multiple modes of disease resistance (Beligni & Lamattina, 2001).

Weight loss of all fruit gradually increased during the storage period; however, the losses were significantly ($p < 0.05$) reduced by the SNP treatments (Figure 4.9B) compared to the control fruit. At the end of the storage period, weight loss in the controls was 13.5%, and the losses from the fruit given the Post SNP and Pre + Post SNP treatments (8.5% and 8.75%) were significantly lower than the control. Exogenous NO treatment has been shown to slow down respiration by interfering with mitochondrial activity (Pareek, 2017). Weight loss was primarily caused by water loss and water loss caused by the respiration rate. A low respiration rate might have contributed to the low rate of weight loss, and reductions in both respiration rates and weight loss have been shown in citrus and pomegranate fruit during storage (Elyatem & Kader, 1984; Valero et al., 1998) after treatment with SNP. Moreover, the decay also influenced fruit weight loss due to the cell collapse and soaking.

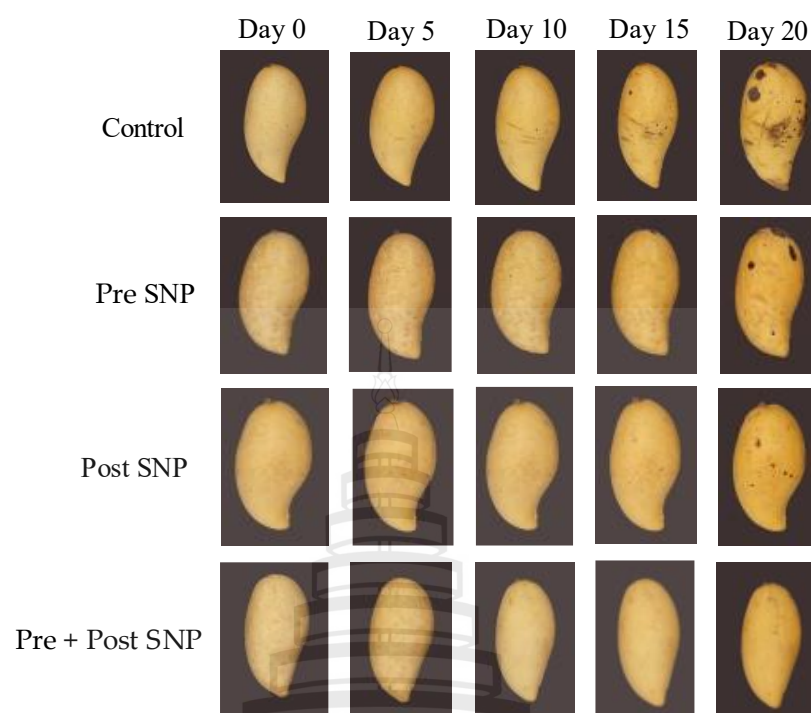
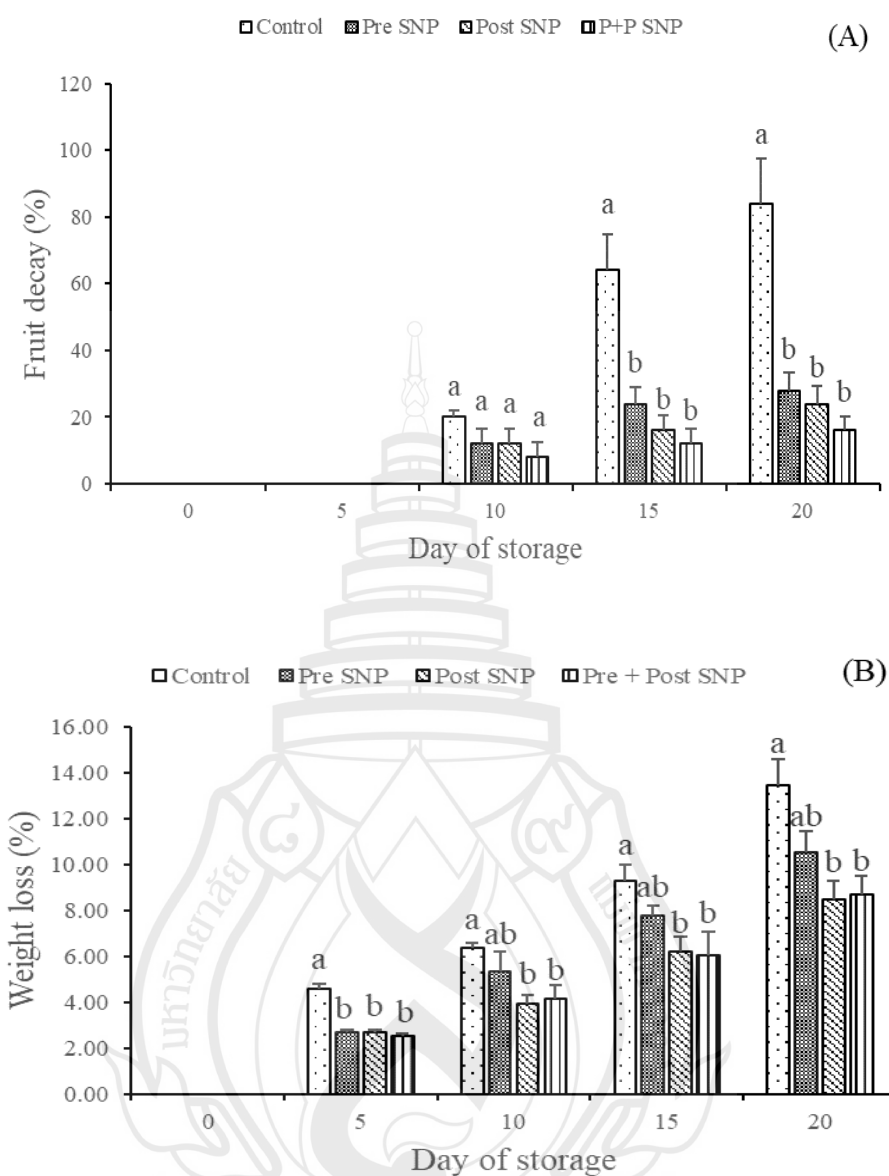


Figure 4.8 The visual appearance of control and SNP-treated fruits during storage at 13°C for 20 days

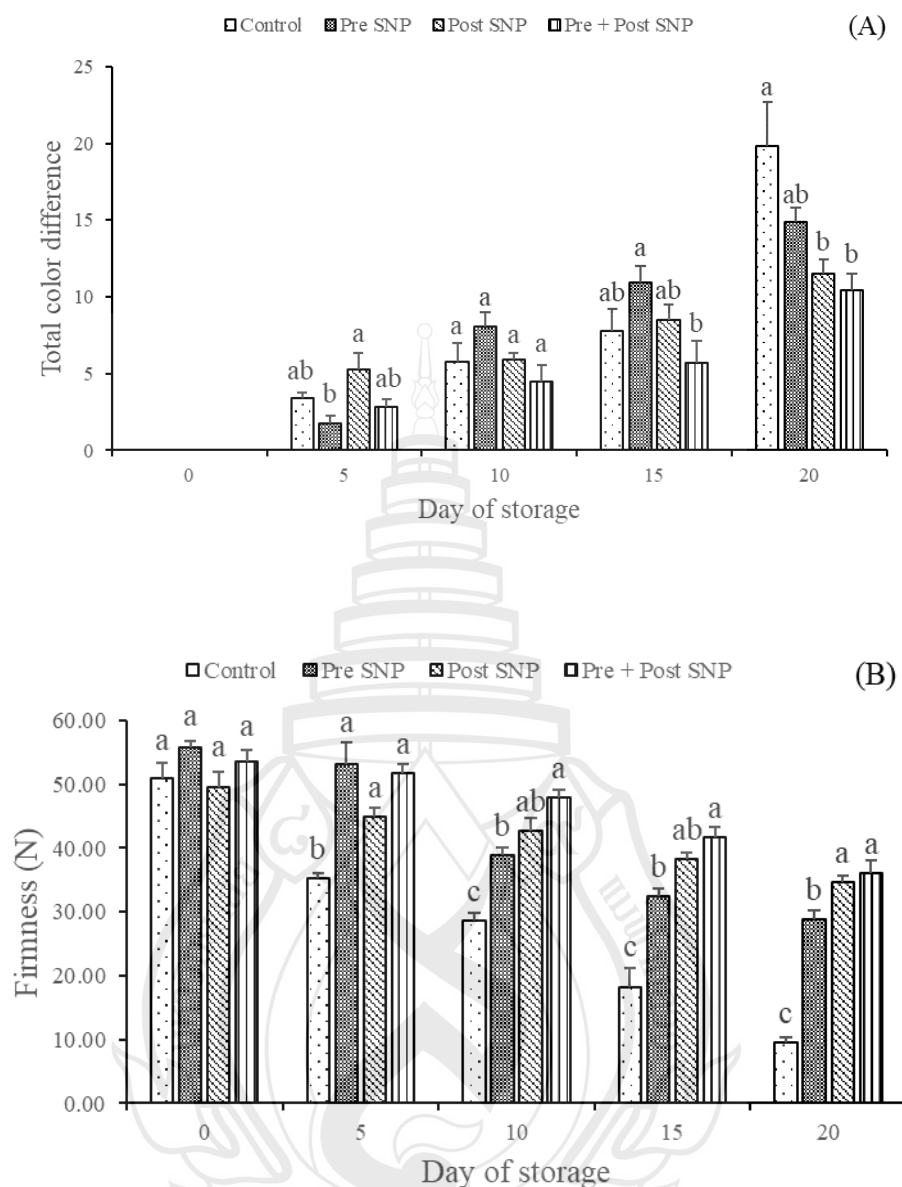


Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.9 Effect of SNP application on (A) fruit decay and (B) weight loss (%) of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

Both the control and treated fruits showed a gradual increase in ΔE (Figure 4.10 A) during storage up to Day 15. However, on Day 20, the control fruit showed a large rise in ΔE , and this color value was statically higher than for fruit treated with SNP. On Day 20, the lowest values occurred in the fruit given postharvest dips of SNP. The changes occur due to chlorophyll degradation and the synthesis of color pigments; similar changes have been found in ‘Guifei’ mango (Hu et al., 2014).

Throughout storage, the fruit in all treatments softened; however, the rate of softening was greatest for the controls. From Day 5 onwards, fruit treated with SNP were significantly firmer than control fruit (Figure 4.10 B), and fruit given postharvest dips were significantly firmer than those only given the pre-harvest spray; however, the fruit in this latter treatment were still significantly firmer than those in the control treatment. Similar effects on firmness have been found in a range of other species including papaya (Li et al., 2014), kiwifruit (Zhu et al., 2010), peach (Flores et al., 2008), and plum (Singh et al., 2009). Fruit firmness is often influenced by the strength of the cell wall and by internal water pressure (Luza et al., 1992), and this study has demonstrated lower weight losses due to treatment with SNP which, at least in part, may be due to decreased water loss. SNP has been shown to reduce the activities of pectin methylesterase, polygalacturonase and cellulase in pear fruit (Adhikary et al., 2021) thereby maintaining fruit firmness.

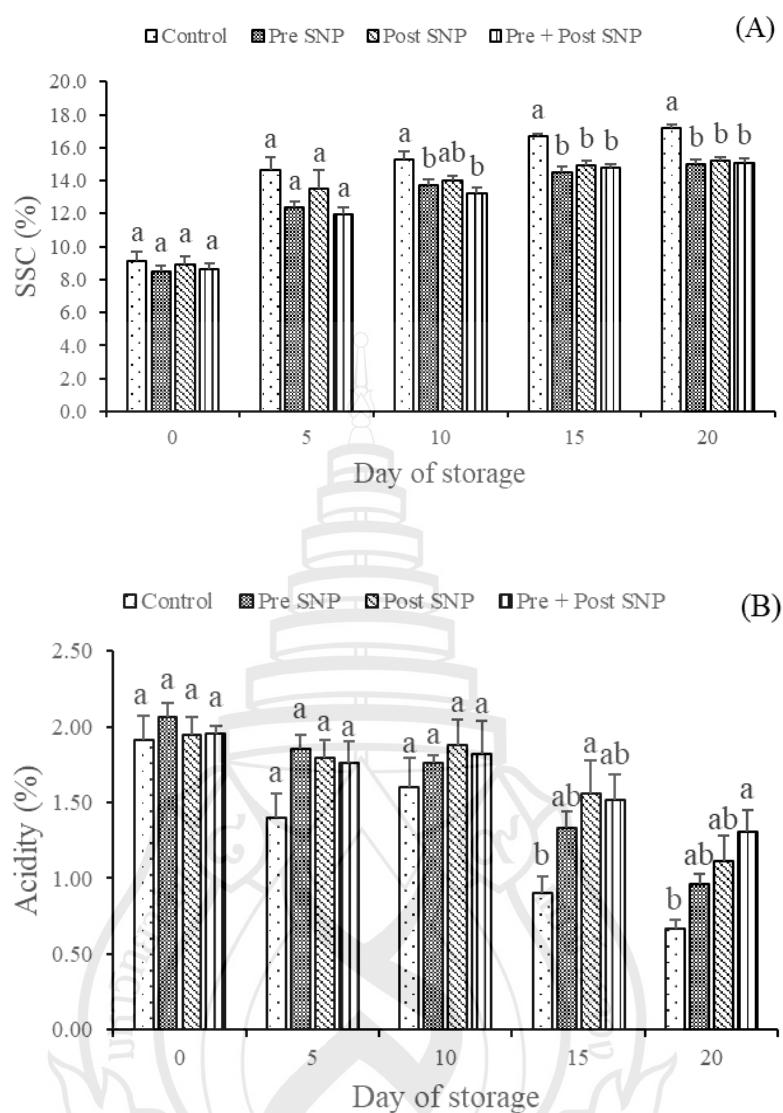


Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.10 Effect of SNP application on (A) Total color difference and (B) Firmness of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

Significant differences ($p < 0.05$) in SSC between control and treated fruits was found from Day 10 onwards (Figure 4.11A) with SNP-treated fruit having lower values; however, there were no significant differences among the SNP treatments. Similar effects on SSC due to SNP application have also been reported in ‘Nam Dok Mai No.4’ mango fruit (Tran et al., 2015). Adhikary et al. (2021) and Chen et al. (2019) reported that SNP sustain SSC in pear and apple fruit, respectively. The latter study ascribed the effects on sugar concentrations to increases in the activities of sucrose phosphate synthase and sucrose synthase synthesis and reductions in the activities of sucrose synthase-cleavage, neutral invertase and acid invertase, and changes in these enzymes in mango warrant further investigation.

Up to Day 10, there were no significant differences in the TA between control and SNP-treated mango fruit at each of the three assessment times and little difference between assessment times (Figure 4.11B); however, differences became apparent in the later assessments. On day 20, the treated fruits showed significantly higher ($p < 0.05$) TA than the control fruits at of ripening. The average TA of the controls was $0.67\% \pm 0.06$ and that of Pre + Post SNP treatment fruits was the highest ($1.31\% \pm 0.14$). The maintenance of acidity in fruits treated with SNP have been caused by a slower conversion of organic acids, as has been suggested by Maftoonazad et al. (2008).



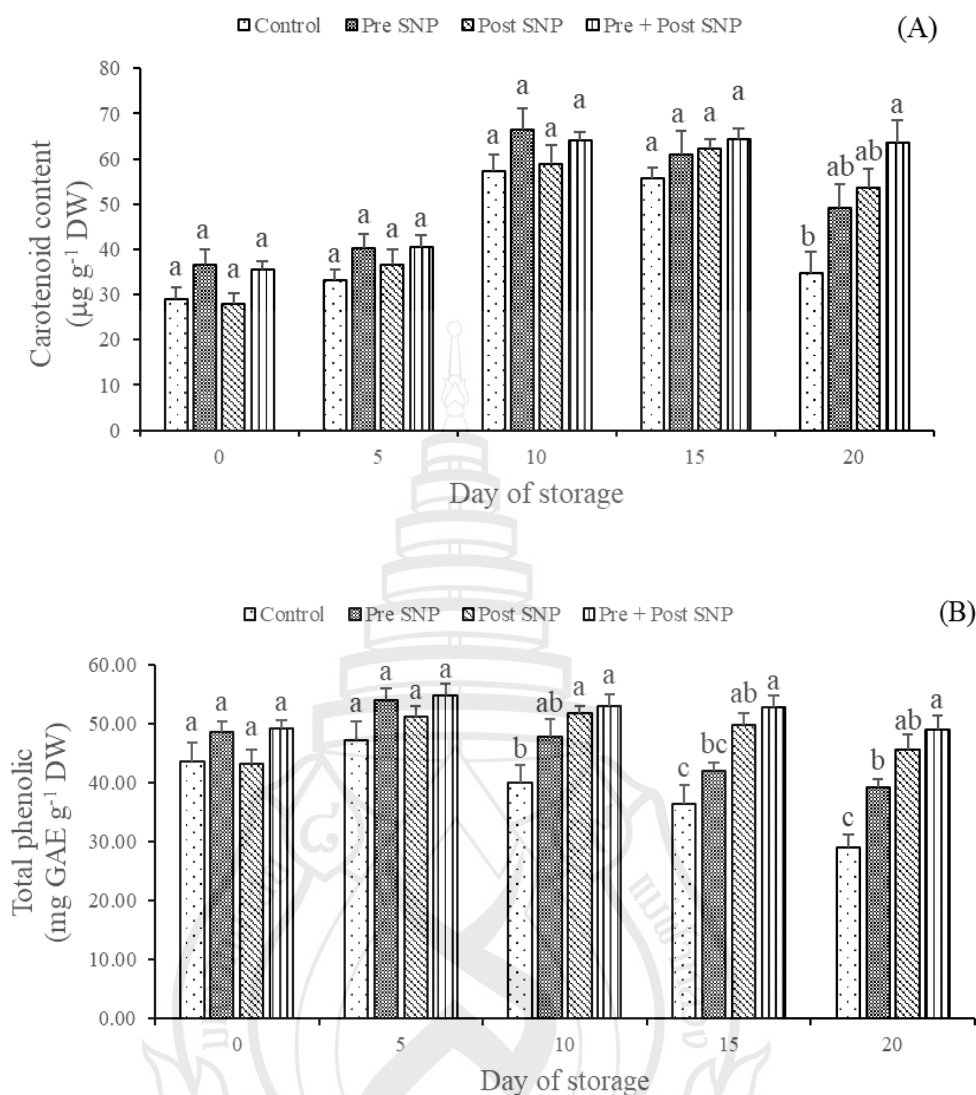
Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.11 Effect of SNP application on (A) soluble solids content and (B) acidity of ‘Nam Dok Mai Si Thong’ mango fruit during storage at 13 °C

4.2.2 Bioactive Compounds

The carotenoid content of control fruit gradually increased from Day 0 to Day 15 and then markedly declined on Day 20 (Figure 4.12A). This reduction was either less in the fruit given the Pre SNP and Post SNP treatments and did not occur in those given the combined treatment. Flores et al. (2008) reported that NO application did not influence the total carotenoid content of peach fruits and slowed or reduced their accumulation in guava (Sahu et al., 2020), mango (Zaharah & Singh, 2011). In this experiment, Pre + Post SNP maintained the carotenoid content until the end of storage. Thus, Pre + Post SNP could preserve nutritional quality in mango fruits.

The TPC of control fruit decreased from Day 10 onwards (Figure 4.12B) and from Day 15 were significantly lower than those of fruit given treatments with SNP. From Day 15, reductions in TPC also occurred in the SNP-treated the magnitudes of which were in the order Pre SNP > Post SNP > Pre + Post SNP. As a result, fruit given the Pre + Post SNP showed little change in these compounds during the assessment period. Phenolic compounds can delay the oxidation of lipids by preventing the initiation or development of oxidative chain reactions (Pennycooke et al., 2005). As a result, the amount of phenolic compounds in mango appears to have a significant impact on the antioxidant activity (Ma et al., 2011).

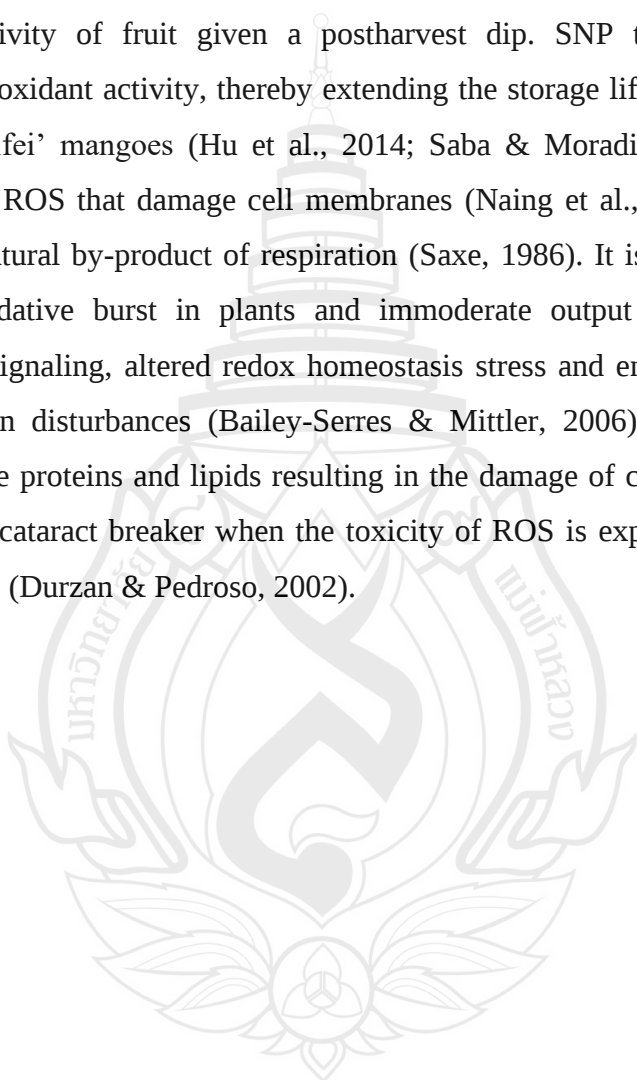


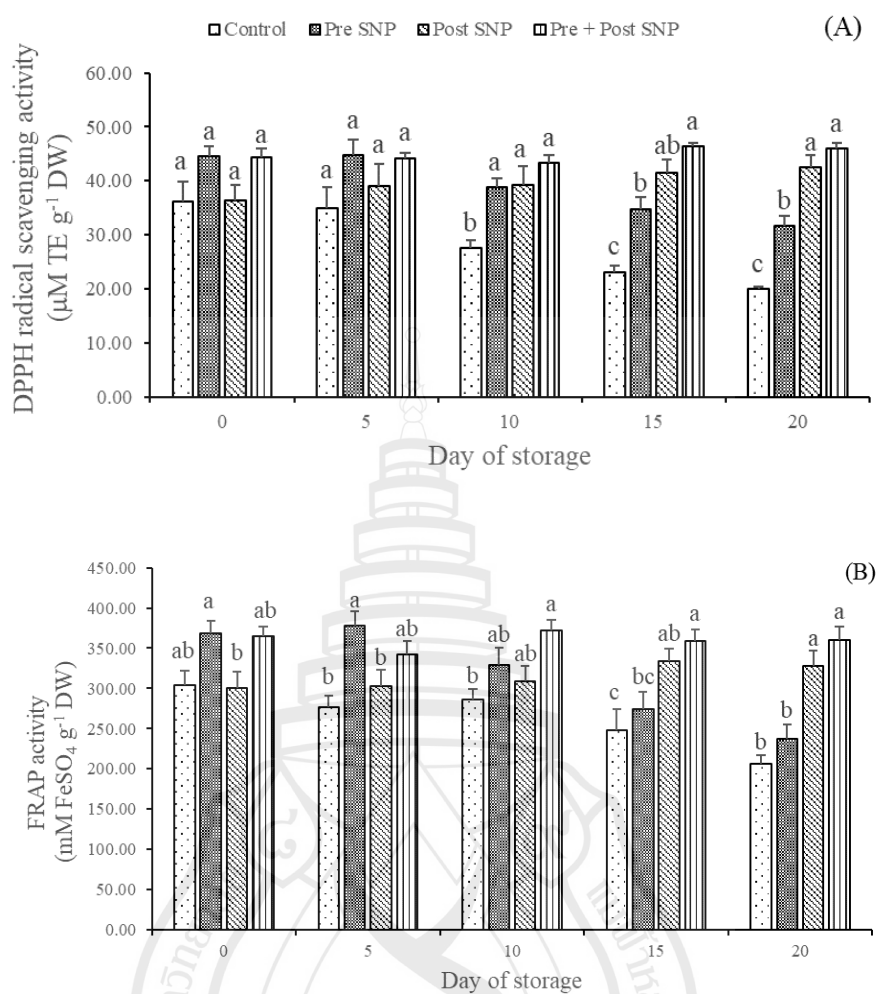
Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.12 Effect of SNP application on (A) carotenoids and (B) total phenolic compounds of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

4.2.3 Antioxidant Activity

The patterns of DPPH radical scavenging activity and FRAP activity were similar with both indices showing a faster decline in the control fruit than in those given the SNP treatments. As with TPC, reductions in TPC also occurred in these two indices the magnitudes of which were in the order Pre SNP > Post SNP > Pre + Post SNP. As a result, at the end of storage period, there was little or no change in antioxidant activity of fruit given a postharvest dip. SNP treatment, therefore, maintained antioxidant activity, thereby extending the storage life as has been shown before for ‘Guifei’ mangoes (Hu et al., 2014; Saba & Moradi, 2017) and acts by scavenging the ROS that damage cell membranes (Naing et al., 2017). ROS can be considered a natural by-product of respiration (Saxe, 1986). It is also said that ROS involve in oxidative burst in plants and immoderate output of ROS results in abnormal cell signaling, altered redox homeostasis stress and enormous coordinated cellular function disturbances (Bailey-Serres & Mittler, 2006). Overexpression of ROS may injure proteins and lipids resulting in the damage of cellular integrity. NO may react as a cataract breaker when the toxicity of ROS is experienced and finally prevent damage (Durzan & Pedroso, 2002).





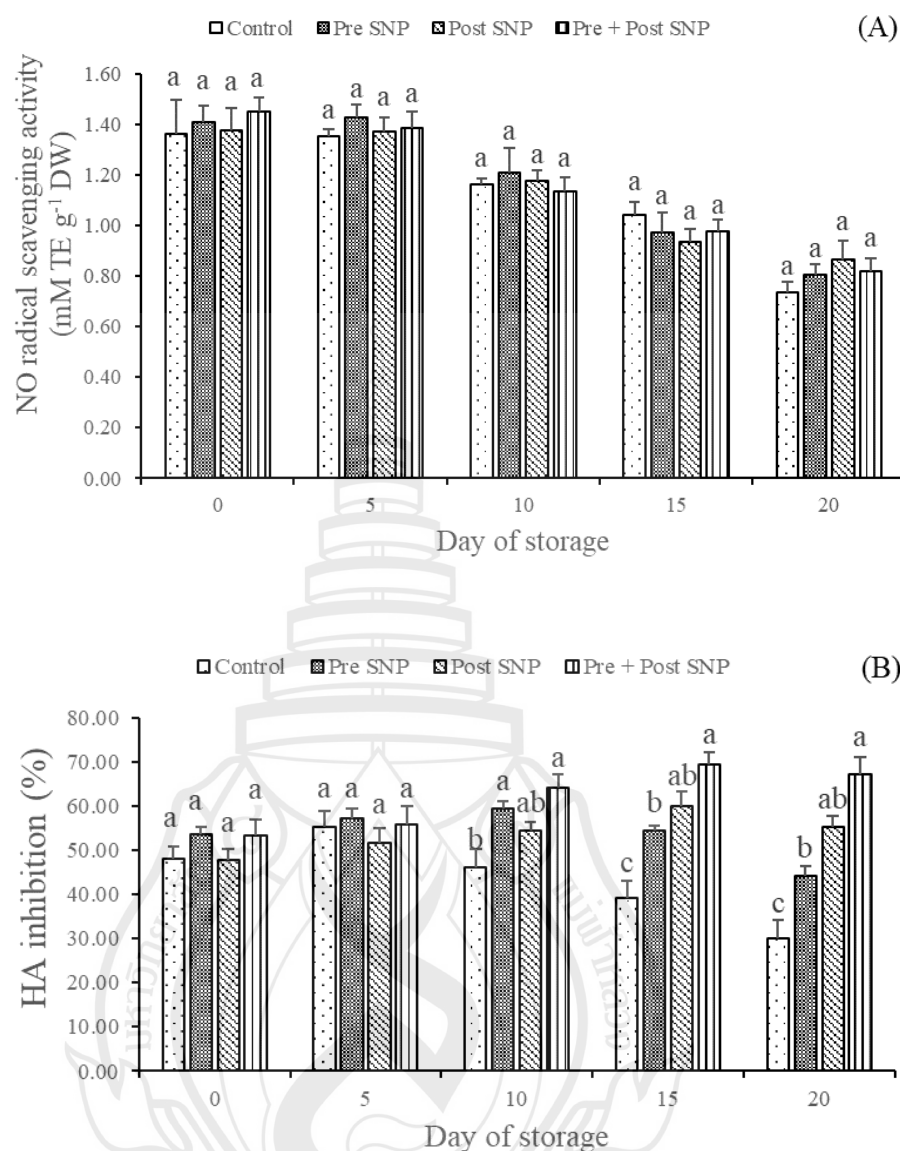
Note Vertical bars represent the standard errors of the means ($n = 5$). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.13 Effect of SNP application on (A) DPPH radical scavenging activity and (B) FRAP activity of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

4.2.4 Anti-inflammatory Activity

Even though NO is a crucial signaling molecule that mediates a variety of physiological and developmental processes in horticulture crops both pre-harvest as well as postharvest, it is a potentially toxic and comparatively unstable free radical gas (Pareek, 2017); high concentrations of NO can result in harmful effects for the treated fruits. In this experiment, SNP treatments were applied and then measured for NO radical scavenging activity of mango fruits to know the effective and safe amount of SNP (NO-donor). In both the control and SNP-treated fruits, NO radical scavenging activity of all fruit decreased during storage (Figure 4.14A); however, there was no significant difference between control and treated fruit at any assessment time. This result shows that neither pre- or postharvest treatments with SNP, nor their combination influence NO radical scavenging activity.

There were no differences between the control and SNP-treated fruit up to Day 5 in hyaluronidase inhibition (Figure 4.14B). After this time, significant differences ($p < 0.05$) between control and SNP-treated became evident with inhibition in the controls declining rapidly, in the Pre SNP treatment declining slowly, whilst inhibition in the Post SNP remained constant and in the combined treatment increasing slightly. The mechanism of NO on HA inhibition activity needs further study. However, according to Bralley et al. (Bralley et al., 2008), sorghum bran extracts that had high total phenolic and antioxidant (FRAP) values inhibited hyaluronidase in vitro more efficiently than extracts with lower phenolic contents. Thus, exogenous application of SNP can increase the hyaluronidase inhibition by enhancing TCP and FRAP activity.



Note Vertical bars represent the standard errors of the means (n = 5). Bars annotated with different letters indicate statistically significant differences among treatments at each storage time

Figure 4.14 Effect of SNP application on (A) NO radical scavenging activity and (B) HA inhibition of 'Nam Dok Mai Si Thong' mango fruit during storage at 13 °C

CHAPTER 5

CONCLUSION

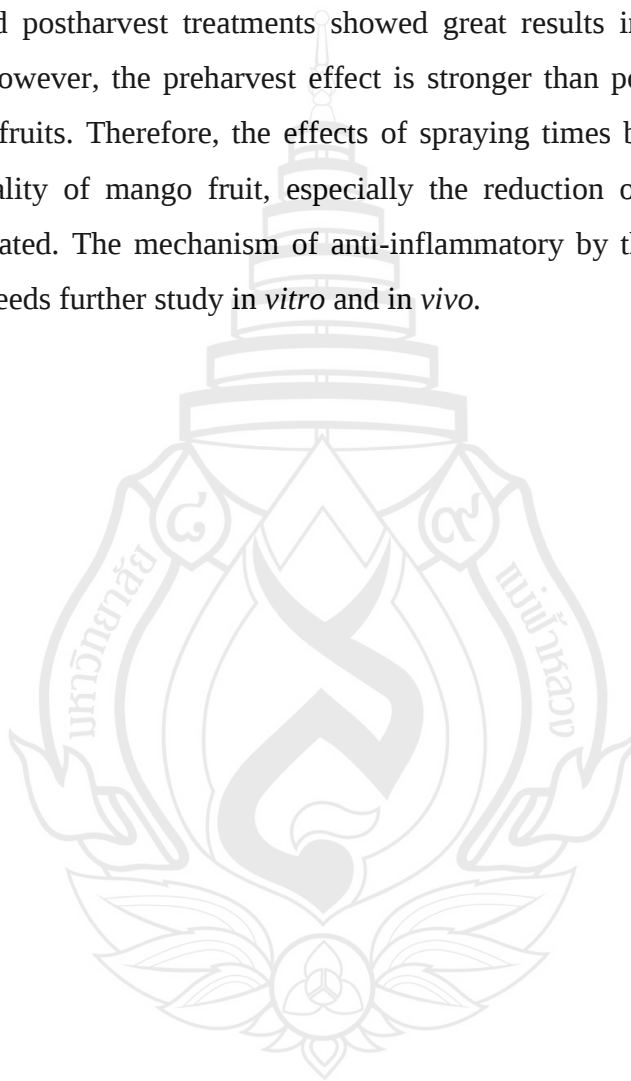
The present study concluded that SA is an effective way of slowing the ripening process thereby maintaining the quality of mango fruit during storage resulting in reducing weight loss and fruit decay, while delaying changes in total color differences, firmness, SSC, and TA. It also enhanced the TPC, antioxidant activity, and anti-inflammatory properties of the fruit. Among the SA treatments, the Pre + Post SA treatment showed the greatest effect on firmness, TSS, TA, FRAP, NO radical scavenging activity and HA inhibition.

For SNP application, SNP treatment delayed ripening including total color differences, firmness, SSC, and TA, as well as reduced weight loss and decay. In addition, the Pre + Post SNP treatment more effectively maintained TPCs, antioxidant activity and HA inhibition more than either the Pre SNP and Post SNP treatments. Thus, this approach can be used to enhance the postharvest quality and storage period. Moreover, it also provides consumers with mango fruit that have better nutritional properties, such as higher contents of bioactive compounds, antioxidants, and anti-inflammatories.

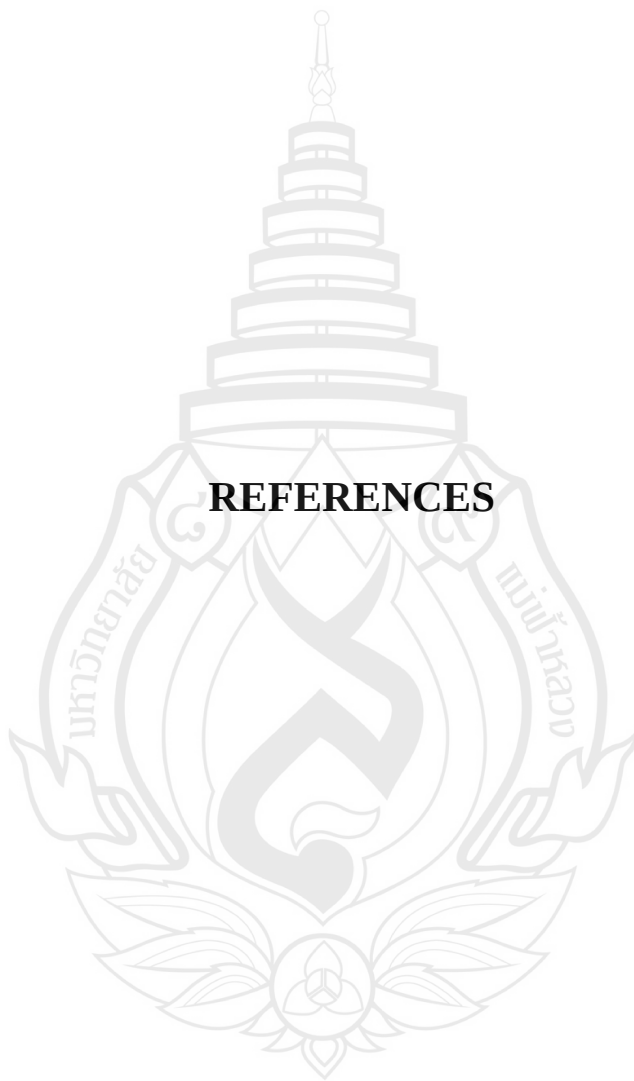
CHAPTER 6

SUGGESTIONS

Pre- and postharvest treatments showed great results in both SA and SNP experiments. However, the preharvest effect is stronger than postharvest, especially for SA-treated fruits. Therefore, the effects of spraying times before harvest on the postharvest quality of mango fruit, especially the reduction of decay, need to be further investigated. The mechanism of anti-inflammatory by the application of SA and SNP also needs further study *in vitro* and *in vivo*.



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